Aloha! Welcome to Hilo and welcome to the 6th International Workshop on Angiostrongylus and Angiostrongyliasis! This is truly an international group with participants coming from Australia, China, Czech Republic, New Zealand, Philippines, Taiwan, Thailand, and the USA. It is my great honor and privilege to welcome all of you here.

I would like to take this opportunity to thank all the people who have helped make this meeting a reality. We could not have run this meeting without the assistance of Professor Gordon Ching. His boundless energy and determination were key in making this happen. We thank the undergraduate pre-med students who are assisting during the meeting. I would also like to thank the members of my lab who have contributed and helped organize including Lisa Kaluna, Dr. John Jacob, Dr. Argon Steel, Yaeko Tagami, and especially Kirsten Snook who was at the other end of all those emails!

I also thank the funding agencies including Hawai‘i County, Hawai‘i State Legislature, Hawai‘i Department of Health, Hilo Medical Center, Malama O Puna, Gordon Ching and community members, and NIH INBRE for their generous support. We also thank individual Hawai‘i Council members Ashley Lehualani Kierkiewicz, Matt Kaneali‘i-Kleinfelder, Maile Medeiros David, and Rebecca Villegas for their generous funding support.

We provide you with a very diverse group of presentations that are on the cutting edge of Angiostrongylus research. I would like to express my sincere gratitude and appreciation to all of the guest speakers and participants for their invaluable contributions to this event. Please look for the special issue of Parasitology with John Ellis as Editor in the near future.

Last but not least, I wish you three days of insightful and enriching sessions and a great stay in Hilo.

Mahalo!

Sue Jarvi, PhD.
Professor
Department of Pharmaceutical Sciences,
Daniel K. Inouye College of Pharmacy
University of Hawaii at Hilo
Mahalo for your interest in Rat Lungworm Disease research! This summary explains the subjects that will be discussed at the Workshop, in order. This will help you determine which talks you are most interested in attending.

Day 1: The Workshop will commence with a talk by a survivor who contracted rat lungworm disease in early 2019, and his experience being officially diagnosed and treated on Hawai‘i island. We hope this will help our clinicians in their practice to understand the patients’ experience of exposure, diagnosis, and treatment, showing where the system is working – and where improvements may be made.

From there, we will begin our clinical discussion of the disease in humans. Case studies and presentation of the disease, diagnostics, and treatment will be discussed.

Once these talks are concluded, the afternoon will be veterinary talks. Case studies, detection, and treatment of rat lungworm disease in domestic and companion animals will be discussed.

Day 2: We will begin the day with a discussion of the genetic similarities and differences between rat lungworm (Angiostrongylus cantonensis) and closely related species, as well as similarities and differences in the genetic makeup of A. cantonensis in different regions of the world. These genetic differences may provide important clues about differences in the presentation of disease and detection of disease in different regions, as well as the transmission cycle of the parasite.

From there we will begin to discuss epidemiological aspects of the disease. Epidemiology is the study of how diseases spread in populations, and analysis of factors contributing to disease.

After epidemiology, we begin to examine the intermediate host (slugs and snails), and transmission dynamics of the disease. How are people infected? What factors contribute to the spread of disease in the definitive host (rat) and the intermediate host (snailslug)? What role do paratenic (accidental transport) hosts play in the spread of disease, if any? We will hear about what happens to the infective larvae in the environment, about finding it in wildlife in Hawaii, and risk factors for agricultural workers.

After this section, we will move into the prevention and education session. How do consumers at home protect themselves and their gardens? Is water catchment a factor, and how do we make sure our catchment systems are safe? How do growers make sure they are not exporting slugs and worms? How do we teach our keiki to be safe and protect them from this disease?

This will conclude our presentations.

Day 3: This will be an opportunity for researchers to discuss what they’ve heard over the first two days and ask each other questions about where the research needs to go from here – where are the gaps in knowledge that we need to fill, and how do we best fill them? This collaborative session may result in new studies, new avenues of inquiry, and revisiting questions about this enigmatic disease that have not yet been answered.

Preventing angiostrongyliasis is our collective kuleana. With knowledge and research, we can protect ourselves and our families. Mahalo for your support, and for attending this Workshop.
## Program schedule for the 6th International Workshop on *Angiostrongylus* and Angiostrongyliasis

### Sunday, 5 January 2020

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>2:00 pm to 5:00 pm</td>
<td>Early check-in</td>
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### Monday, 6 January 2020

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>7:00 am to 8:20 am</td>
<td>Check-in</td>
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<tr>
<td>8:30 am to 9:00 am</td>
<td>Welcome by Dr. Sue Jarvi. Opening remarks by Lieutenant Governor Josh Green, MD.</td>
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<tr>
<td>9:00 am to 9:20 am</td>
<td>Clinical Session Begins.</td>
</tr>
<tr>
<td>9:20 am to 9:40 am</td>
<td>Chairs: Dr. Vernon Ansdell and Dr. Praphathip Eamsobhana.</td>
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<tr>
<td>9:40 am to 10:00 am</td>
<td>Presentation: Mark LeRoy and Maya Parish (USA)</td>
</tr>
<tr>
<td>10:00 am to 10:20 am</td>
<td>Surviving Rat Lungworm Disease: Front Line Perspectives of a Patient and Caregiver</td>
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<tr>
<td>10:20 am to 10:40 am</td>
<td>Presentation: Dr. Vernon Ansdell (USA)</td>
</tr>
<tr>
<td>10:40 am to 11:00 am</td>
<td>Presentation: Dr. Chad Meyer (USA)</td>
</tr>
<tr>
<td>10:40 am to 11:00 am</td>
<td>Chronic Neuroangiostrongyliasis, a neglected aspect of the disease, with long term case reviews from Hawaii, and recommendations for both retrospective and ongoing studies</td>
</tr>
<tr>
<td>10:00 am to 10:20 am</td>
<td>A systematic review on treatment of eosinophilic meningitis</td>
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<tr>
<td>10:20 am to 10:40 am</td>
<td>A clinical review of traditional and alternative therapies in the treatment of <em>Angiostrongylus cantonensis</em> infection</td>
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<tr>
<td>10:40 am to 11:00 am</td>
<td>Coffee/tea break</td>
</tr>
<tr>
<td>10:40 am to 11:00 am</td>
<td>Resume clinical session. Chairs: Dr. Jon Martell and Dr. Kittisak</td>
</tr>
</tbody>
</table>
11:00 am to 11:20 am
Presentation: Dr. Vernon Ansdell (USA)
*Angiostrongylus cantonensis* (Neuroangiostrongyliasis) in travelers

11:20 am to 11:40 am
Dexamethasone downregulates the expressions of MMP-9 and oxidative stress in mice with eosinophilic meningitis caused by *Angiostrongylus cantonensis* infection

11:40 am to 12:00 pm
Sandwich dot-immunogold filtration assay (DIGFA) for specific immunodiagnosis of active neuroangiostrongyliasis

12:00 pm to 12:20 pm
Serologic detection (ELISA) of *A. cantonensis* exposure in humans using a 31 kDa antigen isolated from Hawai’i Island nematodes

12:20 pm to 12:40 pm
Pharmaceutical intervention of *A. cantonensis*: in vitro drug tests

12:40 to 2:20
Lunch

2:20 pm to 2:40 pm
Further studies of neuroangiostrongyliasis in Australian dogs: 78 new cases (2010-2019)

2:40 pm to 3:00 pm
Magnetic Resonance Imaging findings in 5 dogs with neuroangiostrongyliasis

3:00 pm to 3:20 pm
First documented cases of canine neuroangiostrongyliasis due to *Angiostrongylus cantonensis* in Hawaii

3:20 to 3:40 pm
Coffee/tea break

3:40 pm to 4:00 pm
Confirmed cases of RLWD in dogs of East Hawai’i

4:00 pm to 4:20 pm
Real-time PCR detection of *Angiostrongylus cantonensis* DNA in peripheral blood of domestic animals

4:20 pm to 4:40 pm
Gross, microscopic, radiologic, echocardiographic and haematological findings in rats experimentally infected with *Angiostrongylus cantonensis*

4:40 pm to 5:00 pm
Presentation: Dr. Barbora Feckova (Czech Republic)
Pathology of *Angiostrongylus cantonensis* infection in model bird species

Conclusion of Veterinary Session.

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<tr>
<th>Time</th>
<th>Event</th>
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<tr>
<td>5:00 pm to 6:00 pm</td>
<td>Break</td>
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<tr>
<td>6:00 pm to 8:00 pm</td>
<td>Poster session: Biomedical research at University of Hawaii (heavy pupus/appetizers provided; no-host bar)</td>
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**Tuesday, 7 January 2020**

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<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>8:30 am to 8:40 am</td>
<td>Day 2: Remarks by Dr. Susan Jarvi</td>
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<tr>
<td></td>
<td>Genetics and Epidemiology Session.</td>
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<td></td>
<td>Chairs: Dr. Robert Cowie and Dr. Jan Šlapeta</td>
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<tr>
<td>8:40 am to 9:00 am</td>
<td>Presentation: Dr. Robert Cowie (USA)</td>
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<tr>
<td></td>
<td>How many species of <em>Angiostrongylus</em> are there?</td>
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<tr>
<td>9:00 am to 9:20 am</td>
<td>Presentation: Dr. Jan Šlapeta (Australia)</td>
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<tr>
<td></td>
<td>The mitochondrial genome of <em>Angiostrongylus mackerrasae</em> is distinct from <em>A. cantonensis</em> and <em>A. malaysiensis</em></td>
</tr>
<tr>
<td>9:20 am to 9:40 am</td>
<td>Presentation: Dr. Shan Lv (PR China)</td>
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<td>Global Epidemiology of Angiostrongyliaisis: a Systematic Review</td>
</tr>
<tr>
<td>9:40 am to 10:00 am</td>
<td>Presentation: Dr. Noppadol Aekphachaisawat (Thailand)</td>
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<td>A web-based surveillance model of eosinophilic meningitis: future prediction and distribution patterns</td>
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<tr>
<td>10:00 am to 10:20 am</td>
<td>Presentation: Dr. Kittisak Sawanyawisuth (Thailand)</td>
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<td>An ecological study on eosinophilic meningitis caused by <em>Angiostrongylus cantonensis</em></td>
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<tr>
<td>10:20 am to 10:40 am</td>
<td>Coffee/ tea break</td>
</tr>
<tr>
<td>10:40 am to 11:00 am</td>
<td>Presentation: Dr. Heather Walden (USA)</td>
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<td><em>Angiostrongylus cantonensis</em> in Florida, USA: current status</td>
</tr>
<tr>
<td>11:00 am to 11:20 am</td>
<td>Presentation: Dr. David Modrý (Czech Republic)</td>
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<tr>
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<td>The role of L3 larvae released to environment in <em>A. cantonensis</em> transmission</td>
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<tr>
<td>11:20 am to 11:40 am</td>
<td>Presentation: Randi Rollins, MS (USA)</td>
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<tr>
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<td><em>Angiostrongylus cantonensis</em> infection prevalence and intensity in its intermediate gastropod hosts: the impact of host species and environmental variables</td>
</tr>
<tr>
<td>11:40 am to 12:00 pm</td>
<td>Presentation: Lisa Kaluna, MS (USA)</td>
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<td></td>
<td><em>Angiostrongylus cantonensis</em> in slugs and snails of east Hawai’i island and Kauai: a comparison of infection levels with neuroangiostrongyliaisis hotspots</td>
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<tr>
<td></td>
<td>Genetics and Epidemiology Session concludes.</td>
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<tr>
<td>Time</td>
<td>Event</td>
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<tr>
<td>12:00 pm to 1:40 pm</td>
<td>Lunch&lt;br&gt;Transmission Dynamics, Prevention, and Education Session begins.&lt;br&gt;Chairs: Dr. Christopher Niebuhr and Dr. Heather Walden&lt;br&gt; Presentation: Randi Rollins, MS (USA)</td>
</tr>
<tr>
<td>1:40 to 2:00 pm</td>
<td>Species identity and host size are associated with rat lungworm infection in gastropods&lt;br&gt;Presentation: Dr. Christopher Niebuhr (New Zealand)</td>
</tr>
<tr>
<td>2:00 pm to 2:20 pm</td>
<td>Investigations into rat lungworm (<em>Angiostrongylus cantonensis</em>) infections in wildlife populations in Hawai`i&lt;br&gt;Presentation: Dr. Vachel Gay Paller (Philippines)</td>
</tr>
<tr>
<td>2:20 pm to 2:40 pm</td>
<td>Intensive farming: implication for zoonotic parasite emergence&lt;br&gt;Presentation: Dr. Lorrin Pang (USA)</td>
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<tr>
<td>2:40 pm to 3:00 pm</td>
<td>An effective electrified metal barrier to control <em>P. martensi</em>&lt;br&gt;Presentation: Dr. Argon Steel (USA)</td>
</tr>
<tr>
<td>3:00 pm to 3:20 pm</td>
<td>Coffee/ tea break&lt;br&gt;Presentation: Lindsay Hamilton, MS (USA) X-ray irradiation for control of semi-slugs and rat lungworm disease&lt;br&gt;Presentation: Lisa Kaluna, MS (USA) Efficacy of rainwater catchment sediment filters against infective (L3) <em>A. cantonensis</em> larvae, a pilot study&lt;br&gt;Presentation: Yaeko Tagami (USA)</td>
</tr>
<tr>
<td>3:40 pm to 4:00 pm</td>
<td>Mortality rates of the infective (L3) stage <em>Angiostrongylus cantonensis</em> larvae following ultraviolet-C (UVC) irradiation&lt;br&gt;Presentation: Dr. Argon Steel (USA) Evaluation of prospective vegetable washes for killing <em>A. cantonensis</em> larvae&lt;br&gt;Presentation: Kay Howe, MS (USA) Engaging students in citizen science through the prevention of rat lungworm disease&lt;br&gt;Transmission Dynamics, Prevention, and Education Session concludes.</td>
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**Wednesday, 8 January 2020**

<table>
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<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>8:30 am to 10:20 am</td>
<td>Round Table Discussion: Clinical (Dr. Vernon Ansdell moderating) and Veterinary (Dr. Richard Malik moderating)</td>
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<tr>
<td>10:20 am to 10:40 am</td>
<td>Coffee/ tea break&lt;br&gt;Round Table Discussion: Genetics, Transmission Dynamics, Epidemiology, Ecology, Prevention, and Education (Dr. Rob Cowie and Dr. Chris Niebuhr moderating)</td>
</tr>
<tr>
<td>10:40 am to 11:50 am</td>
<td>Closing discussion and remarks</td>
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A web-based surveillance model of eosinophilic meningitis: future prediction and distribution patterns

Noppadol Aekphachaisawat¹, Kittisak Sawanyawisuth¹, Chetta Ngamjarus³
¹Silpakorn University, Bangkok, Thailand
²Sleep Apnea Research Group, and Department of Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
³Khon Kaen University, Khon Kaen, Thailand

Background: Web-based surveillance is a useful tool for predicting future cases of various emerging infectious diseases. There are limited data available on web-based surveillance and patterns of distribution of eosinophilic meningitis (EOM), which is an emerging infectious disease in various countries around the world.

Methods: This study applied web-based surveillance to the prediction of EOM incidence and the analysis of its distribution pattern by using a national database, which may be used for future prevention and control. The number cases of EOM in each month over a period of 12 years (between 2006 to 2017) from Loei province were retrieved from the National Disease Surveillance (Report 506) website, operated by Thailand’s Public Health Center.

Results: We developed autoregressive integrated moving average (ARIMA) models and seasonal ARIMA (SARIMA) models. The best model was used for predicting numbers of future cases. The forecast values from the SARIMA (1, 1, 2)(0,1,1)6 model were close to actual values and were the most valid, as they had the lowest RMSE and AIC. The predictive model for future cases of EOM was related to previous numbers of EOM cases over the past eight months. The disease exhibited a seasonal pattern during the study period.

Conclusions: Web-based surveillance can be used for future prediction of EOM, that the predictive model applied here was valid, and that EOM exhibits a seasonal pattern.

Key words: Angiostrongylus cantonensis, epidemiology, seasonal
Vernon Ansdell MD, FRCP, DTM&H

Associate Clinical Professor, Department of Tropical Medicine, Medical Microbiology and Pharmacology. John A Burns School of Medicine, University of Hawaii, Honolulu, Hawaii, USA.

- University of Birmingham Medical School, United Kingdom (MB, ChB), 1970
- Diploma of Tropical Medicine and Hygiene, Liverpool University, United Kingdom, (DTM&H), 1974
- Member of the Royal College of Physicians of Edinburgh, UK (MRCP), 1976
- Fellow of the Royal College of Physicians UK (FRCP), 1999
- Member, Governor’s Task Force on Rat Lungworm Disease (Neuroangiostrongyliasis)
- Chair, Subcommittee on Clinical Management, Governor’s Task Force on Rat Lungworm Disease (Neuroangiostrongyliasis)

Dr. Ansdell practiced and taught at the London School of Tropical Medicine and Hygiene and the Hospital for Tropical Diseases in London for many years and has a special interest in tropical diseases. In addition, he has a particular interest in leptospirosis, seafood poisonings, marine envenomations and neuroangiostrongyliasis. He is currently working on national, evidence-based guidelines for the diagnosis and treatment of neuroangiostrongyliasis.

Recent Publications:


Ansdell, V.E.: Food Poisoning from Marine Toxins, In CDC Health Information for International Travel (The Yellow Book) 2018, Oxford University Press, 2018

Ansdell VE, Yupaporn W. Angiostrongylus cantonensis in travelers: clinical manifestations, diagnosis, and treatment. Current Opinion in Infectious Diseases. 31, 399-408, 2018

Preliminary Guidelines for the Diagnosis and Treatment of Human Neuroangiostrongyliasis (Rat Lungworm) in Hawaii. Clinical Subcommittee of the Hawaii Governor’s Task Force on Rat Lungworm Disease, 2018


1. Angiostrongylus cantonensis (Neuroangiostrongyliasis) in Travelers

Vernon Ansdell 1, Johnnie Yates 2, Kenton Kramer 1, William Gosnell 1

1 Department of Tropical Medicine, Medical Microbiology & Pharmacology, University of Hawaii, Honolulu, Hawaii, USA
2 Department of Tropical and Travel Medicine, Hawaii Permanente Medical Group, Honolulu, Hawaii, USA

Angiostrongylus cantonensis is a globally emerging infectious disease. It is increasingly recognized as an important infection in travelers from nonendemic to endemic areas of the world. Important factors include increased travel to tropical and subtropical destinations and more adventurous eating habits in travelers. Climate change may also promote spread of the disease from its current tropical and subtropical range into more temperate regions of the world. As a result, we can anticipate more cases of disease in travelers At least 82 cases in travelers have been reported worldwide. Undoubtedly, many cases are unrecognized or underreported. Most cases are in travelers from nonendemic countries to endemic countries, but infection can occur within the same country when there is travel from nonendemic to endemic regions. The majority of published cases have been in travelers from nonendemic areas of Europe and the US mainland who became infected whilst traveling in SE Asia, the Pacific Islands and the Caribbean. Limited awareness in nonendemic areas will result in delays in diagnosis and treatment likely leading to adverse outcomes. Travelers to Angiostrongylus endemic areas should avoid potentially contaminated foods containing raw or undercooked intermediate or paratenic hosts. Salad vegetables and fruits should be carefully inspected and washed prior to consumption. In developing countries raw salad vegetables should probably be avoided altogether because of the additional risk of other pathogens. It is essential that practitioners advising travelers familiarize themselves with the epidemiology, clinical features, diagnosis and management of neuroangiostrongyliasis. They should identify travelers to endemic areas prior to travel and counsel them appropriately. A detailed, travel and food history should be obtained from returned travelers from endemic areas if they develop any relevant symptoms. In suspected cases of neuroangiostrongyliasis prompt treatment with high dose corticosteroids and anthelmintics such as albendazole is often recommended.
2. *Angiostrongylus cantonensis* Infection from Ingestion of Contaminated Kava.

Vernon Ansdell ¹, Kenton Kramer ¹, William Gosnell ¹, Elizabeth Blalock ², Johnnie Yates ³, Jourdan Posner ¹, Pakieli Kaufusi ¹

¹ Department of Tropical Medicine, Medical Microbiology & Pharmacology, University of Hawaii, Honolulu, Hawaii, USA
² Department of Neurology, Spark M Matsunaga VA Medical Center, Honolulu, Hawaii, USA
³ Department of Tropical and Travel Medicine, Hawaii Permanente Medical Group, Honolulu, Hawaii, USA

A unique cluster of cases of neuroangiostrongyliasis occurred in April 2017 when 6 individuals became ill after drinking kava contaminated by a dead slug. Investigation confirmed *Angiostrongylus cantonensis* infection in 3 patients. The remaining 3 patients were considered to be probable infections. All patients were male with a mean age of 36 years (range 26-52 years). There was a wide range of symptoms. All 6 patients experienced headaches but only 2 had low grade fever. Five patients described muscle aches or myalgias. Three patients had nausea or vomiting, abdominal pain, limb pains or some form of sensory symptoms (eg paresthesias or hyperesthesias). One patient had muscle weakness. One patient described generalized pruritus without a rash. Bowel or bladder dysfunction and cranial nerve palsies were not present in any of the patients. Five patients had significant peripheral eosinophilia (absolute eosinophil count > 0.6). Lumbar puncture (LP) was performed on 4 patients only as it was technically not possible in one patient due to extreme obesity and one patient declined the procedure. One patient required repeat LP to relieve persistent headaches. Significant cerebrospinal fluid (CSF) eosinophilia (>10% or >10 eosinophils/cu mm) was present in 3 of the 4 patients. There were 2% eosinophils of 160 white blood cells /cu mm in the remaining patient. Diagnosis was confirmed in 3 patients by a positive real-time polymerase chain reaction (RTi-PCR) test for *A. cantonensis* DNA of CSF. Mean time to starting treatment with corticosteroids was 5 days after symptom onset (range 2-8 days). All 6 patients were treated with high dose corticosteroids with 5 patients additionally treated with albendazole. High cost prevented the use of albendazole in the 6th patient. All 6 patients appeared to recover without residual signs or symptoms.
Dr. Cowie’s research interests since moving to Hawaii in 1990 have been diverse, focusing on both native and alien biodiversity, specifically but not exclusively on non-marine snails. This has involved research on biogeography, conservation, systematics and biodiversity. Since 2004 he began surveys of alien snails (which includes slugs) in Hawaii and have documented numerous new introductions. These surveys involved collecting large numbers of specimens from sea level to mountain-top across the six largest islands. Live specimens preserved in ethanol were thus available to address the presence of Angiostrongylus cantonensis (rat lungworm) throughout the Islands and across the diversity of snail species. He and his team demonstrated that 16 of 37 species screened carried rat lungworm. Those that did not may have been collected from habitats unsuitable for the parasite or simply by chance were not infected. They also showed that snail species from across the entire gastropod phylogenetic tree can act as hosts. They developed habitat suitability models that identify the potential range of the parasite in the Hawaiian Islands and extrapolated its potential range in 2100 using various climate change scenarios. In 2011, he organized an invited workshop (now considered the 2nd workshop in the series of international workshops on Angiostrongylus and angiostrongyliasis) to address research priorities related to angiostrongyliasis, to which he invited international participants including parasitologists, epidemiologists, physicians, public health professionals, researchers developing diagnostic tools, and snail biologists (herself and students). This workshop introduced him to the global community of Angiostrongylus professionals. He co-organized the follow up meeting in Guangzhou in 2013 and participated as an invited speaker in the meeting in Bangkok in 2017. Most recently he has developed collaborations with Angiostrongylus researchers at the Universities of Sydney, Leeds and Belfast. He serves on the Hawaii State Governor’s Rat Lungworm Disease Task Force on the basis of his expertise involving the intermediate snail hosts. He has a broad understanding of rat lungworm disease but especially of the underlying biology of the transmission cycle of the parasite, which is the area of research that he is currently pursuing with my students and collaborators. He is also interested in the nomenclature and systematics of angiostrongyliids in general, having recently published a major review.

Recent publications (those related to Angiostrongylus asterisked)


*Odani, J.S., Cowie, R.H., Malik, R. & Jarvi, S.I. In review. Rat lungworm disease for the veterinary professional. *Veterinary Extension, College of Tropical Agriculture and Human Resources, University of Hawaii 2.*


Angiostrongylus Baylis, 1928, two in has been fluid. At one extreme, Ubelaker, in 1986, recognized only three species in Angiostrongylus. Dougherty considered Angiostrongylus (Baillet, 1866) and a new species, Kamensky in 1905 with two included species, Kamensky, 1905. By 1946, A. vasorum Angiostrongylus (family Angiostrongylidae, superfamily Metastrongyloidea, order Strongylida) was established by E Mail cowie@hawaii.edu Pacific Biosciences Research Center, University of Hawaii, Honolulu, Hawaii 96822, USA

Robert H. Cowie

How Many Species Of Angiostrongylus Are There?

The genus Angiostrongylus (family Angiostrongylidae, superfamily Metastrongyloidea, order Strongylida) was established by Kamensky in 1905 with two included species, A. vasorum (Baillet, 1866) and a new species, A. cardicus Kamensky, 1905. By 1946, Dougherty considered Angiostrongylus to include seven valid species. Since then, additional angiostrongylid genera have been established, with numerous new species. However, acceptance of the validity of these genera and placement of species in them has been fluid. At one extreme, Ubelaker, in 1986, recognized only three species in Angiostrongylus, placing 12 in Parastongylus Baylis, 1928, two in Angiocaulus Schulz, 1951, three in Gallegostrogylyrus Mas-Coma, 1977, one in Rodentocaulus Schulz, Orlov & Kutass, 1933, and three in Stefaskostrongylus Drozdz, 1970. At the other extreme, Bhaibulaya and Cross, in 1971, recognized 13 species in Angiostrongylus, with four in Stefaskostrongylus, yet by 1991 Bhaibulaya placed all of these, plus three species more recently described, in Angiostrongylus, for a total of 20 species. Recent lists (2012-2019) – there have been no major taxonomic revisions – include 18-23 species in Angiostrongylus. A rigorous nomenclatural analysis of all species that at one time or other have been included in Angiostrongylus, reflecting the most consistent placements of species in this and other genera, leads to a total of 21 in Angiostrongylus, four in Gallegostrogylyrus, one in Rodentocaulus and ten in Stefaskostrongylus. Three of these are especially well known, A. cantonensis, A. costaricensis and A. vasorum, because of their role in human and animal diseases. Many others are far more poorly known, often only from their original description, and from remote type localities. In many cases the type material has been lost, including that of A. cantonensis and A. vasorum. The published nomenclatural and type catalog that developed this analysis also includes much information about hosts and distributions and provides a rigorous basis for future revisionary study.

Keywords: Angiostrongylus, Gallegostrogylyrus, Rodentocaulus, Stefaskostrongylus, nomenclature, types


Formulation strategies to improve solubility and oral bioavailability of antiparasitic agents

Abhijit A. Date
Department of Pharmaceutical Sciences, The Daniel K. Inouye College of Pharmacy, University of Hawaii Hilo, Hilo, HI 96720

The oral bioavailability of majority of antiparasitic agents is limited due to their poor aqueous solubility and/or permeability which eventually affect their clinical efficacy. Our work is focused on the modulation of solubility of anthelmintic benzimidazoles (albendazole, mebendazole and flubendazole), macrocyclic lactones (ivermectin and moxidectin) and praziquantel to improve their oral bioavailability. Here, we present preliminary data on the development of clinically viable formulation approaches such as micelles and lipid-based formulations to improve oral delivery of various antiparasitic agents. We anticipate that the novel anti-parasitic formulations being developed in our research group will have applicability for the treatment of a variety of parasitic infections including rat lung-worm disease.
Professor Dr. Praphathip Eamsobhana
Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University

Praphathip Eamsobhana holds a Bachelor of Science with Honors in Medical Technology from Chulalongkorn University, Master of Science in Tropical Medicine from Mahidol University, and PhD in Immunology from University of Malaya. She obtained post-graduate diplomas in Medical Parasitology from University of Queensland, and Applied Parasitology and Entomology with Distinction (Top student) from IMR-SEAMEO-TROPMED, Kuala Lumpur, Malaysia. Prof. Praphathip is former Chair of the Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, where she is active in teaching and scientific research that focuses on various food-borne zoonotic parasites, particularly immunology, molecular biology, systematics and genetics. She has published over 100 research articles, of which over 80 relate to the rat lungworm Angiostrongylus cantonensis, and nine chapters in books and monographs. She has written two books on the rat lungworm, Angiostrongylus cantonensis – parasitology, immunology, eosinophilic meningitis, epidemiology, laboratory diagnosis (2006), and The Rat lungworm Angiostrongylus cantonensis – parasitology, genetics and molecular phylogeny (2015). She also works very closely with the Department of Medical Sciences and Department of Disease Control, Ministry of Public Health, Thailand in the promotion of public understanding and awareness on the rat lungworm and its disease.

Dr. Praphathip is recognized internationally for her research publications on the rat lungworm disease and laboratory diagnostics. Some of her research findings have direct applications in clinical and field practices worldwide. She was among the pioneers to adopt a holistic and innovative approach to solving problems in immunodiagnosis for eosinophilic meningitis/meningoencephalitis caused by A. cantonensis which is endemic in the Pacific islands and Southeast Asia, including Thailand (especially in the northeast region where the local populace enjoy eating a popular raw snail dish called “koi-hoi”). Her noteworthy achievement and contribution is the finding of a 31 kDa glycoprotein antigen of A. cantonensis specific for the diagnosis of human angiostrongyliasis cantonensis and unequivocal differentiation of eosinophilic meningitis due to other clinically related helminth infections, e.g. cerebral gnathostomiasis, neurocysticercosis, cerebral paragonimiasis. In addition, the diagnostic test kits that she had developed with the 31-kDa antigen (dot-blot ELISA, multitot-ELISA, AcDIGFA, AcQuick™), have proven to be rapid, simple, effective and economical for clinical use and some are particularly suited for large scale screening in the field. In addition, her contribution and achievement are not confined to just one aspect of the parasites but covered many others relating to the parasites. For example, she studied not only the diagnostics of the rat lungworm and other related helminths, but also biochemical and molecular systematics, parasites and vectors relating to the worm, control measures, geographical distribution, diversity, etc. Her genetic and systematic approaches of Angiostrongylus cantonensis and allied species is particularly outstanding. Her first publication on molecular phylogenetics using cytochrome c oxidase subunit I (COI) as a gene marker for differentiation of Angiostrongylus species and geographical isolates of A. cantonensis had led to many similar papers from other groups of scientists worldwide using various gene markers for the genetic differentiation in addition to phylogenetic study.

Recent Publications


Sandwich Dot-Immunogold Filtration Assay (Digfa) for Specific Immunodiagnosis of Active Neuroangiostrongyliasis

Praphathip Eamsobhana¹, Anchalee Tungtrongchitr¹, Hoi-Sen Yong²,
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Serological tests may yield false-negative results for specific antibodies detection before or early seroconversion phase with Angiostrongylus cantonensis infection. Tests that detect circulating antigens of A. cantonensis would therefore be of value in individual diagnosis to distinguish current or past infection. A rapid, easy to perform, sensitive and specific test for detection of 31-kDa A. cantonensis specific antigens had been developed, based on the rapid flow-through immunoassay. Rabbits were individually immunized with soluble adult worm antigen and purified 31-kDa glycoprotein antigens of A. cantonensis to produce immune sera. The sandwich dot-immunogold filtration assay (“AcDIGFA”), for detecting active angiostrongyliasis was produced using anti-A. cantonensis polyclonal antibody coated on nitrocellulose membrane as a capture agent and colloidal gold-labeled anti-31 kDa A. cantonensis antibody as a detection agent. A well-defined pink dot, indicating positivity, was seen readily by naked eye within 10-15 min. The visual detection limit detected by the AcDIGFA test was approximately 0.01 µg mL⁻¹. Diagnostic accuracy of AcDIGFA test for the detection of 31-kDa A. cantonensis specific antigens was initially evaluated using cerebrospinal fluid (CSF) samples from two confirmed cases with cerebral gnathostomiasis and one confirmed case with neurocysticercosis. The present vertical flow-through AcDIGFA test enables rapid qualitative detection (presence/absence) of the specific 31-kDa antigens of A. cantonensis without complicated steps, and with good diagnostic sensitivity and specificity - thus it has potential for practical use in rapid diagnosis of early/active A. cantonensis infection, even under resource-limited settings. Further evaluation with a greater panel of clinical CSF/serum samples for its diagnostic value is warranted and is ongoing.

Keywords: Angiostrongylus cantonensis, sandwich dot-immunogold filtration assay, DIGFA, antigen detection, 31-kDa antigen, active angiostrongyliasis
Barbora Fecková  
Postgraduate Student

- Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences in Brno, Czech Republic., Doctor of Veterinary Medicine (MVDr.), 06/2017, Veterinary medicine
- Department of Pathological Morphology and Parasitology, University of Veterinary and Pharmaceutical Sciences in Brno, Czech Republic, PhD, Currently enrolled, Veterinary parasitology, Dr. Feckova is a hard-working postgraduate student in the field of Veterinary Parasitology, working on the biology of metastrogylid nematode infections. She has spent over 9 months at internships in Spain and Romania, learning about wildlife parasitology and pathology, and a month as a volunteer veterinarian in the Cheetah Rescue Centre Somaliland. Communicative in English and Spanish. Her career goal is a scientific work focused on zoonotic parasites, species conservation and education.

She has spent the last two years studying the biology and distribution of Angiostrongylus cantonensis in-situ (Canary Islands and Philippines) and by means of experimental work and molecular analysis. In 2019, she presented a talk, "Angiostrongylus cantonensis in birds and other wildlife", and led a workshop titled “Detection of Angiostrongylus cantonensis in its definitive and intermediate hosts” at the Wildlife Diseases and Conservation Medicine symposium at University of Philippines, Los Baños, Philippines. In 2018, her grant proposal “Pathology of Angiostrongylus cantonensis infection in model bird species” was accepted by the Internal Grant Agency UVPS Brno.

Recent publications


Pathology of Angiostrongylus cantonensis infection in model bird species

Barbora Fecková¹, Michaela Valašťanová², Michaela Petríková³, Zdeněk Knotek⁴, Zora Knotková⁵, David Modrý⁶
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Angiostrongylus cantonensis is known to infect wide range of warm-blooded animals that serve as accidental hosts. Clinical cases of neural angiostrongylosis are described in several bird species, while consistently higher number is reported from Australia in tawny frogmouths Podargus strigoides. This led us to speculate that A. cantonensis is a significant pathogen with possible effect on populations of susceptible bird species. Laboratory isolate of A. cantonensis from French Polynesia, genotyped as clade 2 using sequences of COI gene, was used to assess the effect of experimental infection in chicken and Japanese quail. L3 larvae were isolated from experimental Subulina octona snails by pepsin-HCl digestion. First group of birds was infected by 100 L3 larvae p.o., second group was infected by 1500 L3. The birds in third group were fed three intact individuals of S. octona, mimicking natural infection; two laboratory rats were infected as positive control. Birds were observed during one-week period and euthanized afterwards. Clinical signs, hematology, biochemistry and necropsy and histology findings were used to assess the pathology of the infection. Subtle neurological signs were detected in three out of nine infected chickens, quails remained without observable health alteration. Hematology in all infected experimental groups, except for negative control, showed changes in lymphocyte, heterophil and eosinophil counts. No major gross lesions were observed during necropsy; histopathology revealed subtle meningitis in three quails, however, larvae were not observed in any of inoculated birds. Our results did not prove susceptibility of galliform birds to A. cantonensis infection. The dose of 1500 L3 is often lethal for rodents of similar or higher body weight, yet it produced only mild clinical symptoms in few of the experimental birds. This opens a question of using domestic galliform birds as pest control in endemic areas, lowering the number of gastropods carrying the infective larvae.

Keywords: birds, experimental, neurology, pathology, angiostrongylosis

Themes: experimental infections
Lindsey Hamilton  
**Biological Science Technician**
- University of Hawaii at Hilo, Hilo, HI, BA, 12/2007, Biology (Conservation, Ecology and Evolution)  
- Evergreen State College, Olympia, WA, MS, 12/2015, Environmental Studies

Lindsey has a broad background in conservation biology, natural resource management, and quantitative biological research. Her studies of the natural world started at the University of Hawaii at Hilo and focused on the ecological impacts of invasive species on Hawaii ecosystems. Her early professional experiences include data management, botanical and wildlife monitoring, and native plant production. As a graduate student at The Evergreen State College, she explored the social-political and economic sides of environmental work. She advocated for social justice and conservation while coordinating an endangered butterfly rearing program within a state prison for the Sustainability in Prisons Project. Her thesis research examined nocturnal resource selection and movement of sea ducks in the Salish Sea using satellite telemetry and GIS. She also engaged in multiple partnerships to evaluate the efficacy of various water quality restoration strategies throughout the Puget Sound of Washington State for the Washington State Department of Health.

For the last three years, she has been working at the Daniel K. Inouye Pacific Basin Agricultural Research Center in the Tropical Crop and Commodity Protection Research Unit in Dr. Peter Follett’s lab. She collaborates on research which aims to help farmers with their most challenging pest problems. She has investigated the biology and ecology of the infamous coffee pest, Coffee Berry Borer and evaluated potential post-harvest phytosanitary irradiation treatments for fruit flies.

Currently, she is partnering with Dr. Sue Jarvi’s lab at the University of Hawaii at Hilo, College of Pharmacy, to develop phytosanitary irradiation treatments for the semi-slug Parmarian martensi and A. cantonensis. Effective post-harvest treatments are needed to ensure that exported agricultural products do not result in the establishment of these two species at receiving locations.

**Recent publications**

Hamilton LJ, Hollingsworth RG, Sabado-Halpern M, Manoukis NC, Follett PA, Johnson MA. Coffee berry borer (Hypothenemus hampei) (Coleoptera: Curculionidae) development across an elevational gradient on Hawai‘i Island: Applying laboratory degree-day predictions to natural field populations. PLOS ONE. 2019;14: e0218321. doi:10.1371/journal.pone.0218321


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**X-ray irradiation for control of semi-slugs and rat lungworm disease**


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X-ray irradiation is used to control quarantine insect pests in fresh fruit and vegetables exported from Hawaii to the U.S. mainland. A generic radiation dose of 400 Gy is approved for all insects. Parmanian martensi, an invasive semi-slug, is the prominent vector of Angiostrongylus cantonensis (rat lungworm disease) in Hawaii. It has been found on produce (e.g. sweet potatoes, taro leaves) exported to California, raising concerns. Nematodes can be highly tolerant of irradiation. However, a study in China using rats suggested 500 Gy could prevent development of A. cantonensis. A study was conducted to determine if irradiation at the slightly lower generic insect dose of 400 Gy would also control A. cantonensis. Survivorship of A. cantonensis after x-ray irradiation at doses between 200-600 Gy was evaluated using propidium iodide dye and the operetta imaging system. No signs of mortality after irradiation were detected using this method. Additional studies are needed using rat hosts to determine the possible efficacy of a 400 Gy irradiation treatment against A. cantonensis. In addition, potential post-harvest irradiation treatments need to be evaluated for P. martensi.
Engaging Students in Citizen Science through the Prevention of Rat Lungworm Disease

Rat lungworm disease is a real-life, serious issue for Hawaii, and in particular for Hawaii Island; it has claimed lives, livelihoods, and lifestyles. However, while the disease presents many problems for Hawaii it also provides a surprising number of unique and rewarding opportunities for K-12 STEAM (science, technology, engineering, art, math) education in public health, and strategies for lowering infection rates in host animals and reducing the potential for cases of disease. Inserting rat lungworm disease education into the K-12 system as STEAM learning was recorded as one of the focus priorities for public education to minimize a chance of rat lungworm infection at the 2011 International Rat Lungworm Workshop held in Honolulu, Hawaii. The Jarvi Lab at the University of Hawaii, Hilo Daniel K. Inouye College of Pharmacy has been developing educational materials to be used in K-12 classrooms since 2012. A collaborative effort between the Jarvi lab, individuals, non-profits, and agencies have allowed for the expansion of this work. The overall objective is to provide information via written materials, workshops, and professional development education courses, that train teachers and their students to become proficient citizen scientists and community educators in an effort to reduce the potential for rat lungworm disease transmission. Through the integration of integrated pest management and citizen science activities, and the building of community connections, K-12 teachers engage students in meaningful learning that grows a generation of farmers, gardeners, and consumers who understand the measures that must be taken to prevent infection and control the spread of the rat lungworm parasite. Lessons and activities are intended to expand teacher professional development and increase teacher and student exposure to STEAM subjects and STEAM career tracks.
In vitro efficacy of seven FDA approved anthelmintics on Angiostrongylus cantonensis L3 larvae

John Jacob, Ingo Lange, Ghee Tan and Susan I. Jarvi.
Department of Pharmaceutical Sciences, Daniel K. Inouye College of Pharmacy, University of Hawai’i at Hilo

The nematode Angiostrongylus cantonensis has a lifecycle requiring mollusks as the intermediate host and rats as the definitive host and is the leading cause of eosinophilic meningitis worldwide, with life-threatening complications if not managed correctly. The use of anthelmintic drugs has historically been controversial since the efficacy of anthelmintics on A. cantonensis has not yet been thoroughly established as most of the previous studies were in vivo studies with insufficient controls and clinical case reports. The in vitro studies have utilized the lack of motility of adult female worms to assess the death of A. cantonensis post-treatment, but these results are not directly applicable to a clinical setting, since it is the L3 stage larvae and not the adult worms that are infectious. Additionally, conclusions based on motility can be misleading since inactive or immobile behavior is common among nematodes, regardless of exposure to a test substance. Through differential staining using propidium iodide penetration as the marker of death combined with the nematode’s natural autofluorescence we are now able to distinguish between dead and live nematodes. This assay has enabled us to demonstrate the in vitro sensitivity of seven FDA approved anthelmintic drugs including albendazole sulphoxide, pyrantel pamoate, diethylcarbamazine citrate, levamisole hydrochloride, piperazine, praziquantel and niclosamide ethanolamine (at 1mM concentrations) on A. cantonensis L3 larvae which were isolated from wild Parmarion martensi from east Hawai’i Island. Piperazine, praziquantel and niclosamide showed no significant effects on A. cantonensis L3 larvae even after 30 days post-treatment. In contrast, significant larval staining (mortality) was observed with albendazole, pyrantel pamoate, diethylcarbamazine and levamisole treatments as compared to the DMSO controls (P<0.05). The results of this study indicate the need for evaluation of multiple drug doses and parasite exposure levels at several time points early post-exposure using an animal model.
Jarvi, Susan Irene
Professor, Pharmaceutical Sciences

- Fitchburg State University, MA, B.S., 1983, Education
- University of Massachusetts, Amherst MA, M.S., 1986, Vet & Animal Sciences, Avian Genetics
- Northern Illinois University, DeKalb IL, Ph.D., 1989, Biology, Immunogenetics
- Beckman Research Institute, City of Hope National Medical Center, Duarte, CA, Post-doc, 1990-93, Molecular Genetics
- Molecular Genetics Laboratory, National Zoological Park, Smithsonian Institution, Washington DC, Post-doc, 1993-95, Molecular Evolution

Positions and Employment
2000-2005 Assistant Professor, Biology Department, University of Hawaii at Hilo
2006-2015 Director, Pre-Pharmacy Program, University of Hawaii, Hilo. Establishment and development of the Pre-pharmacy, Pacific Pre-pharmacy (2008) and STEP (2010) Programs at UHH
2006-2009 Chair, Biology Department, University of Hawaii, Hilo
2005-2009 Associate Professor, Biology Department, University of Hawaii, Hilo
2009-2014 Associate Professor, Department of Pharmaceutical Sciences, University of Hawaii, Hilo
2014 –present Professor, Department of Pharmaceutical Sciences, University of Hawaii, Hilo
2015-2016 Department Chair, Department of Pharmaceutical Sciences, University of Hawaii, Hilo

Relevant Publications


Lisa M. Kaluna  
*Research Associate/ Lab Manager*

- University of Hawai‘i at Hilo, Hilo Hawai‘i, BA, 05/2004, Marine Science & Biology  
- University of Hawai‘i at Hilo, Hilo Hawai‘i, MS, 05/2009, Tropical Conservation Biology & Environmental Science

Ms. Kaluna has been a research associate and laboratory manager for Dr. Susan Jarvi at the Daniel K. Inouye College of Pharmacy, University of Hawai‘i at Hilo (UHH) since 2017. Her research endeavors have been focused on two primary areas of rat lungworm disease (1) prevention through the decontamination of water, produce, or fomites by testing the effectiveness of rainwater catchment sediment filters and electromagnetic radiation (UVC, x-ray, and blue light) as well as (2) ecological genetics by testing infection in intermediate and paratenic hosts. She moved to Hilo in 2002 and earned her MS in Tropical Conservation Biology and Environmental Science from UHH investigating the genetic diversity of free-living *Symbiodinium* and horizontal transmission to symbiotic coral larvae. Following her masters, she was awarded a 2010 Knauss Marine Policy Fellowship and placed in the National Sea Grant Office within the National Oceanic and Atmospheric Administration working on sustainable coastal development and hazard resiliency. She returned to the islands and spent six years at Hawai‘i Community College (HawCC) managing the biology lab and teaching introductory biology and human anatomy & physiology courses. From 2014-2018, she was co-coordinator of the National Institute of Health IDeA Networks of Biomedical Research Excellence undergraduate research internship program for the UHH and HawCC campuses with Dr. Jarvi where they initiated a collaboration on rat lungworm disease in 2015.

**Recent publications**


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**1. Efficacy of rainwater catchment sediment filters against infective (L3) *A. cantonensis* larvae, a pilot study**

LM Kaluna1, Howe K1, Lozano A1, Torres Fischer B1, Tagami Y1, McHugh R1, Jarvi S1

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Rainwater catchment systems as the primary domestic water supply are widespread and unregulated in households of Hawai‘i Island. Infected intermediate hosts, such as *Parmarion martensi*, may enter a water storage tank and, upon drowning, release live, infective, third-stage (L3) *A. cantonensis* larvae. The most widely endorsed method for water treatment in the U.S. includes a series of sediment filters followed by a UV disinfection system, however no research exists on its effectiveness against large waterborne pathogens including nematodes. In this pilot study, a model water catchment system was used in a laboratory setting to test the efficacy of five filters in blocking *A. cantonensis* larvae. Filters tested included 20 µm, 10 µm, 5 µm, and 1 µm pore size with either wound polypropylene, spun polypropylene, or carbon block construction. Live nematodes, including infective *A. cantonensis* larvae, were able to penetrate all filters except the 5 µm carbon block. Filters that were unsuccessful in blocking all nematodes did significantly reduce the number of nematodes in post-filtrate water. Catchment users should exercise caution and not expect results of this study to be transferrable to their own catchment systems. Methods used here have limited detection capabilities, small sample sizes, and performed in a laboratory environment with treated municipal water. Filter performance may vary due to debris in the reservoir or on the filter, pressure changes from varying water pump strength or piping sizes, as well as overall system maintenance.

**Keywords:** Rainwater, catchment, water, rat lungworm, sediment filter

**Themes:** prevention, transmission, decontamination
2. *Angiostrongylus cantonensis* in slugs and snails of east Hawai‘i island and Kauai: a comparison of infection levels with neuroangiostrongyliasis hotspots

LM Kaluna, Howe K, Fiedler KD, Severino MG, Botticelli J, Farias ME, Jarvi S

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*Angiostrongylus cantonensis*, the causative agent of neuroangiostrongyliasis, has been confirmed on every island of Hawai‘i except Lanai. The geographical distribution of cases has shifted by island, most of which are now found on Hawai‘i island. Monitoring of intermediate hosts that carry infectious (L3) stages of *A. cantonensis* could have important implications for land owners, farmers, healthcare providers, and public officials. East Hawai‘i island and Kauai represent an interesting comparison opportunity where they have similar environmental habitats and previously identified similar collection site infection levels, yet two neuroangiostrongyliasis hotspots have been identified by the Hawai‘i Department of Health (HDOH) in east Hawai‘i island. One potentially significant difference between these two island regions is the colonization, widespread distribution, and high abundance of the highly infected, invasive * Parmarion martensi* (semi-slug) across east Hawai‘i, and the presumed absence of this species from Kauai due to a lack of anecdotal or official reports. Molecular screening of slug and snail infection was completed on 352 east Hawai‘i samples and 170 Kauai samples found overall prevalence of 65% and 13% respectively in these regions. Infection in 213 *P. martensi* samples from 18 east Hawai‘i sites found 84% of slugs were infected with high variation in parasite loads, both of which showed no temporal or host size trends in initial analyses. Patterns of *P. martensi* infection did show spatial variation of 62% prevalence with a mean 1 L3 per mg tissue parasite load in the Hilo HDOH hotspot and 87% prevalence with a mean 25 L3 per mg tissue parasite loads in the Puna HDOH hotspot. Additional analyses of slug and snail samples from east Hawai‘i and Kauai will be presented, including data highlighting how differences in experimental design and methods can effect conclusions and implications in slug and snail infection studies.

**Keywords:** rat lungworm, Hawaii, semi-slug, invasive species

**Themes:** prevalence, risk
Mark LeRoy is a licensed realtor specializing in the management of luxury vacation rentals along the Kona-Kohala coast. Prior to his real estate career, Mark worked for 20 years in Los Angeles in brand strategy, marketing and design for beloved brands, including Four Seasons Resort at Hualalai, Hyatt Hotels & Resorts, La Brea Bakery, The Coca-Cola Company, and The Coffee Bean & Tea Leaf. Contracting Rat Lungworm Disease in January 2019 changed Mark’s life. The acute phase of the disease was more devastating than anything Mark has ever experienced, and he continues to struggle with chronic pain as a result of the nerve damage he sustained from the disease. Mark was one of the rare victims who actually knew exactly when he had come into contact with a semi slug in his food. He reported that contact to multiple doctors, some of whom were told less than 14 days after contact. Mark sought medical care within the prescribed window to be treated with albendazole, the anti-parasitic medication, which could have drastically altered the course of the disease for him and could have resulted in less or no chronic pain, but the albendazole was never offered during that critical window. Mark shares his story to support the medical community in improving their response to this disease in order to help future victims. Mark lives in Hawi, Hawai’i with his wife Maya, their newborn daughter Zephyr and their dog Ella.

Maya Parish is a Yoga Teacher, Event Producer and new mother and works as the Operations Manager for her husband Mark’s vacation rental and property management portfolio. She ran for Hawai’i County Council’s District 9 seat in 2018 due to her belief that her community and our natural environment deserved a representative who was ready and willing to fight for their health and well being. She is currently serving as the Hawai’i County At-Large Representative on the State Central Committee of the Democratic Party of Hawai’i. Rat Lungworm Disease has had a substantial impact on Maya. Caretaking for Mark during the acute phase of the disease, along with his business, her business, the house and their dog all while newly pregnant was unlike any other time in her life. She was a fierce advocate for Mark at all of his hospital and doctor’s visits, and was shocked and disappointed at the lack of adequate care Mark received. She shares her story to support the medical community in improving their response to this disease in order to help future victims. Maya lives in Hawi, Hawai’i with her husband Mark, their newborn daughter Zephyr and their dog Ella.

Surviving Rat Lungworm Disease, Front Line Perspectives of a Patient & Caregiver

On January 9th, 2019 Mark LeRoy discovered an adult semi slug on his dinner plate among his salad greens, harvested from his home garden in Hawi, HI. He didn’t bite into the slug, but his salad fork grazed it, and it’s likely he ingested at least one juvenile before discovering the adult. Eight days later the first symptoms of Rat Lungworm Disease started to appear, including pressure in his upper middle back, restless legs, skin pain/tenderness, tingling in extremities and neck, and very sore calves.

Mark’s wife Maya Parish, who discovered she was pregnant right after Mark’s symptoms emerged, ate the same salad but didn’t get sick. Mark’s condition grew worse and worse while Maya cared for him during the first trimester of her pregnancy. Maya and Mark brought a document outlining Mark’s initial slug contact and his detailed symptomatology, along with a printed version of the Governor’s joint task force’s Preliminary Guidelines for the Diagnosis and Treatment of Human Neuroangiostrongyliasis to five Emergency Room visits and three doctor’s visits before Mark finally received the care he needed. It was thanks to a family medicine doctor in Waimea, Dr. Will Chapple, who began treating Mark for Rat Lungworm Disease prior to his diagnosis, that Mark was finally admitted to the hospital in desperate condition.

Mark received two lumbar punctures during his hospital visits. Because the first didn’t show an elevated eosinophil count, the Department of Health would not run a PCR test, though the eosinophil count in his blood was elevated. The second, more than a month after contact with the semi slug, registered a 7% eosinophil count, still below the prescribed threshold for PCR testing. PCR testing was finally done anyway, which proved that Mark was indeed a confirmed case.

Come listen to the symptomatology and story of a survivor and his spouse, how Hawai’i’s medical community failed them, and how we might do better for future victims of this disease.
Global Epidemiology of Angiostrongyliasis: a Systematic Review

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The rat lungworm A. cantonensis is the major cause for human eosinophilic meningitis in tropical region. We conduct a systematic review to provide light on the global epidemiological profile and identify the priorities in research and disease control. We searched the publications in PubMed, ISI Web of Knowledge, Science Direct and Embase basically and extended search in some local databases, e.g. Chinese database (CNKI, VIP, Wangfang, CEPS), Japanese database (CiNii, J-Store), SciELO, LILACS and AJOL. We also used the snowball technique to search other related literature. We identified 1,241 articles and extract data for this review. Over 6000 human cases were identified and distributed in 48 countries/territories. Two peaks of occurrence were noted around

Shan LV
Associate Professor
- Stanford University School of Medicine (USA), Visiting scholar, 11/2017, Geographical medicine and infectious diseases
- University of Basel (Switzerland), PhD, 06/2011, Epidemiology
- Chinese Center for Disease Control and Prevention (China), Master in medicine, 06/2006, Epidemiology and statistics
- University of Zhengzhou (China), Bachelor in medicine, 06/2003, Preventive medicine

My recent research is focusing on schistosomiasis and intermediate host Oncomelania hupensis. We accomplished the first national survey on the snail in China. We are using this database and specimens for ecological models and population genetics. I also conducted a systematic review on the epidemiology of angiostrongyliasis cantonensis and the diversity of host recently and will perform the modeling of global transmission with other experts outside China.

Recent publications
1966 and 2006, respectively. One hundred and four deaths were reported, which led to an overall mortality of 1.5%. There were 232 parasitologically confirmed cases leading to the rate of parasite discovery of 3.4%. Up to date, 92 potential definitive host species were examined for the natural infection of rat lungworm, among which 38.0% (35/92) were found naturally infected. A total of 296 mollusk species were examined for natural infection of rat lungworm larvae, among which 54.1% were found naturally infected. Rat lungworm could occur in some non-permissive definitive hosts, including primate, marsupial, horse, dog, fox, bat, bird, and armadillo. Since high diversity and wide range of host species impose infection risk on humans and wildlife particularly in coastal areas, the potential invasion of rat lungworm and the occurrence of infections should be paid attention from different sectors in South Asia, Africa and Central America.

**Keywords:** Angiostrongyliasis; global epidemiology; systematic review
Further Studies of Neuroangiostrongliasis in Australian Dogs: 78 New Cases (2010-2019)

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A total of 78 dogs 7 weeks to 14 years-of-age (median 5 months) were presumptively diagnosed with neuroangiostrongliasis between 2010 and 2019. 58% of dogs were less than 6 months old. The trend was for progressively more cases to be seen each year, culminating in 17 cases in 2019. Cases comprised various pedigree dogs, pedigree hybrids and one crossbred, generally reflecting the commonness of breeds over time. The gender breakdown was 51 males (18 neutered; 33 entire) and 26 females (13 spayed; 13 entire), with gender unrecorded for two dogs. The preponderance of male dogs presumably reflects them being more exploratory and thus more likely to ingest molluscs. Cerebrospinal fluid (CSF) data was variably retrievable. CSF was obtained from the cisterna magna in 65 dogs, from the lumbar cistern alone in six dogs and from both sites in 3 dogs. Cell counts in CSF for the 71 specimens ranged from 2 to 146,150 cells/µL (median 4,470). CSF specimens were typically slightly turbid. The percentage of eosinophils varied from 15% to 98% (median 83.5%). Both cisternal and lumbar CSF specimens were collected from three dogs, and in each of these the nucleated cell count in lumbar CSF was substantially greater than the corresponding cisternal CSF specimen, suggesting inflammation was more severe caudally. 19 CSF specimens were subjected to ELISA testing for antibodies against *A. cantonensis*; 18/19 (95%) tested positive, with titres ranging from 0 to ≥12,800, with a median titre of 3,200, and a geometric mean of 4,631. CSF serology thus confirmed rat lungworm disease in 18/19 (95%) of dogs using a single CSF specimen. 39 CSF specimens were subjected to qPCR testing for *A. cantonensis* nucleic acid but only seven (18%) tested positive. Of these seven, C₇ values were recorded for five specimens, values ranging from 29.6 to 35.2.

**Keywords:** Angiostrongylus cantonensis; dogs; CSF; ELISA; qPCR

**Themes:** Parasitology, Veterinary, Imaging, Pathology
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- University of Dayton, BS, 1969
- Medical College of Georgia, MD, 1974
- Residency Family Medicine, Medical University of South Carolina, 1977
- University of London, London School of Hygiene and Tropical Medicine, MSc, 1992
- University of Hawaii, post baccalaureate degree primary and secondary education, 2001
- Member Clinical Subcommittee of the Hawaii Governor’s Joint Task Force on Rat Lungworm Disease
- Co author Preliminary Guidelines for the Diagnosis and Treatment of Human Neuroangiostrongyliasis (Rat Lungworm Disease) in Hawaii.

Curriculum vitae and research interests
Kaiser Permanente, Wailuku, Hawaii 2001 - 2014  
primary care, family medicine, travel and tropical medicine, type 2 diabetes intervention program

Assistant clinical professor 2012-2014  
Department of Family Medicine & Community Health John A. Burns School of Medicine, University of Hawaii

Public Health Medicine, Maui 2001- 2002 epidemiological investigation & intervention dengue fever outbreak, Hana, Maui

Foreign Service Medical Officer, Department of State 1978 - 1998  
Singapore 1994 - 1998 (Regional responsibilities: Malaysia, Brunei, Papua New Guinea)

(responsibilities: Zimbabwe, Malawi, Botswana) 
collaborated with CDC studies establishing presence of schistosomiasis in Lake Malawi, occupational health care for Zambian employees including Tb prophylaxis intervention program for HIV positive patients

London School of Hygiene and Tropical Medicine 1991- 1992,  
Masters Science, clinical tropical medicine research: urban malaria in sub-Saharan Africa

Dakar, Senegal 1988 - 1991  
(responsibilities: Gambia, Guinea Conakry, Guinea Bissau, Cabo Verde)  
malaria studies w CDC light-trap field samples of mosquitoes in urban Dakar, collaborated with French researchers at Institute Pasteur in HIV-2 studies

Bangkok, Thailand 1984 - 1988  
(responsibilities: Thailand and Laos) visiting physician Chulalongkorn U. Hospital, Bangkok, weekly teaching rounds

Panama City, Panama 1981 - 1984  
(responsibilities: Costa Rica, Nicaragua, Honduras, Belize, El Salvador, Guatemala) staff physician Gorgas Hospital Panama

conducted clinical trials of Japanese encephalitis vaccine epidemiological studies of diarrheal diseases in Kathmandu valley

Chronic Neuroangiostrongyliasis, a neglected aspect of the disease, with long term case reviews from Hawaii, and recommendations for both retrospective and ongoing studies.

Chad Meyer, MD, MSc, DTMH  
Consultant Tropical Medicine, Maui

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Current and past literature of Neuroangiostrongyliasis (NAS) primarily addresses the acute manifestations of the disease. Mention of chronic, continued clinical symptoms is usually limited to brief notes in discussion of severe cases. This report addresses long term residual symptoms in a group of persons who contracted NAS in Hawaii. The data base suggests multiple persons have residual symptoms for many years including sensory dyskinesias, extremity muscle pains, as well as psychological impairments affecting personal relationships and employment. This study summarizes common features of chronic disease, proposes pathogenic mechanisms responsible for prolonged illness, and highlights the frequently unsuccessful endeavors of individuals struggling to find effective treatment. Recommendation is made for recognition of the frequency of chronic NAS, and for both retrospective and ongoing studies of this neglected component of the disease.
Dr. Alfred J. Mina was born in Lihue, Kauai and raised on the Big Island of Hawaii. After graduating from Hilo High School, he obtained a Bachelor of Science in Agriculture and a Bachelor of Arts in Biology at the University of Hawaii-Hilo. In 2000, Dr. Mina graduated with his Doctor of Veterinary Medicine from Washington State University – College of Veterinary Medicine before heading home to Hilo, Hawaii where he’s been practicing small animal, exotics, avian and emergency medicine since he graduated.

Dr. Mina’s special areas of interest are emergency medicine, avian and exotic animal medicine, and orthopedic surgery. Although his patients are mostly dogs and cats, he also provides medical care for the injured endangered birds and birds of prey of Hawaii.

When Dr. Mina is out of the office, he enjoys spending his free time doing animal paintings with acrylics and soft pastels and caring for his own animals.

**Confirmed Cases of RLWD in Dogs of East Hawaii**

Rat lung worm disease (RLWD), caused by *Angiostrongylus cantonensis*, is an emerging infectious disease occurring on the Big Island of Hawaii in humans and animals. The purpose of this study is to find out the occurrence of infection in dogs living on the island. Patients submitted into the study must have a history of exposure to slugs and show clinical signs of neurologic or spinal disease. Upon presentation blood work is collected for in hospital analysis as well as sending to the University of Hawaii-Hilo where they perform PCR to look for the presence of the nematode DNA for a definitive diagnosis. All patients are then placed on dewormers and other medications per protocol to treat their clinical symptoms. Of the 26 dogs that were tested, 12 were positive, 11 were negative, and 3 were inconclusive. Of the 12 that were positive, 10 were not on any heartworm prevention. Of the 12 that were positive, 10 responded to treatment, 1 was humanely euthanized due to a terminal neoplasia unrelated to RLWD, and 1 was humanely euthanized due to failure of response to treatment for RLWD. We are hoping that with further testing we will be able to find diagnostic trends to enable early detection in infected dogs.
David Modrý
Head of Dept. of Parasitology and Pathology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic and Senior Researcher, Biology Center of Czech Academy of Sciences

- University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, DVM, 1993, Veterinary Medicine
- University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, PhD., 1999, Veterinary Parasitology
- University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, Prof., 2010, Infectious Diseases

In general, Dr. Modrý is interested in complexity of parasite-host interactions in the context of ecosystems, particularly the transmission of infections at the human/wildlife/domestic animal interface. In particular, in case of A. cantonensis, using various methodological tools he and his team try to investigate peculiarities of the life cycle in isolated island ecosystems, as well as the risk of introduction into European territory. Currently, he maintains an experimental A. cantonensis strain in his lab.

Recent Publications

The role of L3 larvae released to environment in A. cantonensis transmission

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Angiostrongylus cantonensis is considered a threat to human health in the endemic areas, and despite the cumulating knowledge on its distribution, the ways of infection of humans are not always explained. Beside intentional consumption of mollusks and amphibians and reptiles carrying the L3 larvae in tissues, unintentional ingestion of L3 larvae with food or water cannot be neglected. In series of experiments with laboratory reared mollusks we tested following hypotheses: (1) terrestrial gastropods accidentally drowned in water release significant numbers of viable L3 larvae in short period of time; (2) environmental stress leads to increased excretion of L3 larvae from intermediate hosts; (3) released L3 larvae are able to infect new, susceptible snail hosts following their release from experimentally infected mollusks (intermediasis). We used experimental laboratory isolate of A. cantonensis from French Polynesia, previously genotyped as clade 2 using sequences of COI gene; experimental molluscs were infected by L1 larvae shed in feces of laboratory rats. We evaluated release of L3 larvae from two species of experimentally drowned slugs of the genus Veronicaella and Subulina octona snails (hypothesis 1) and showed significant differences among the gastropod species used. Furthermore, we tested and quantify the L3 larvae released from experimental Pomacea (hypothesis 2) and, last but not least, we tested the intermediasis in an experimental system involving Pomacea, Veronicaella and Subulina (hypothesis 3).
Chris Niebuhr
Vertebrate Ecologist
- Texas A&M University, Texas, USA, BSc, 12/2003, Wildlife & Fisheries Sciences; Entomology
- Tarleton State University, Texas, USA, MSc, 05/2012, Wildlife Management
- University of Otago, New Zealand, PhD, 05/2016, Wildlife Disease Ecology
- USDA APHIS WS National Wildlife Research Center, Hawai’i Field Station, Hilo, Hawaii, USA, Post-doc, 12/2018, Research Biologist (vertebrate pest & rat lungworm research)

Chris N. Niebuhr is a research scientist for Manaaki Whenua – Landcare Research in Lincoln, New Zealand. As a vertebrate ecologist, he leads research efforts in conservation biology, invasive species management, and disease ecology in New Zealand and elsewhere in the Pacific. He received his BSc with a double major in wildlife & fisheries sciences and entomology from Texas A&M and his MSc in wildlife management from Tarleton State University where he studied ticks’ ecology and their wildlife hosts. He earned his PhD from the University of Otago in New Zealand investigating transmission dynamics of avian malaria. As a postdoctoral research biologist for the USDA National Wildlife Research Center Hawaii Field Station in Hilo, his research focused on invasive rats and mongooses in island ecosystems. While now based permanently in New Zealand, he continues to collaborate with researchers in Hawaii, Australia, and elsewhere on investigations into the epidemiology of rat lungworm disease.

Recent publications

Investigations into rat lungworm (Angiostrongylus cantonensis) infections in wildlife populations in Hawai’i

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Angiostrongylus cantonensis, or the rat lungworm, is a tropical and subtropical parasitic nematode that causes neural angiostrongyliasis (rat lungworm disease) in humans, domestic animals, and wildlife. The lifecycle of the parasite requires both gastropod intermediate hosts and rodent definitive hosts, but can sometimes also involve paratenic (transport) hosts. In Hawai’i, recent reports of high A. cantonensis infection rates in both intermediate (slugs) and definitive hosts (rats), as well as an increase in reported cases in accidental hosts (humans and dogs), have led to an increase in public awareness. Here we present an overview of ongoing collaborative research being conducted on Hawai’i Island investigating transmission dynamics of A. cantonensis in vertebrate host populations. Current findings include infection level results from a large survey of wild rats (Rattus spp.) that suggest acquired immunity to A. cantonensis may be occurring in R. rattus over time after exposure, but not in R. exulans; and observations on temporal differences in infection levels in rats sampled at four seasonal increments in 2018-19, results of which could have important management implications. Additionally, we discuss ongoing work investigating the role of paratenic hosts in transmission in Hawai’i. Recently we have observed infections in two reptile, three amphibian (including the invasive coqui frog, Eleutherodactylus coqui), and one centipede species, all of which may be acting as reservoirs to which disease spillback can occur. Overall, these findings increase our collective understanding of A. cantonensis transmission dynamics and risk of exposure in Hawai’i and elsewhere.
First documented cases of canine neuroangiostrongyliasis due to Angiostrongylus cantonensis in Hawaii

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Two young dogs domiciled in Honolulu, Hawaii were presented in November and December 2018 (respectively) for spinal hyperesthesia, hindlimb weakness, and proprioceptive ataxia. Both dogs had neurologic findings referable to spinal cord disease, with upper and lower motor neuron signs, and hindlimb conscious proprioceptive deficits. Peripheral eosinophilia was present in the second case, but hematology and serum biochemistries were otherwise unremarkable. Plain radiographs and computed tomography scans with and without contrast were unremarkable. Cerebrospinal fluid (CSF) from both patients demonstrated eosinophilic pleocytosis, and real-time PCR (qPCR) testing demonstrated Angiostrongylus cantonensis DNA in CSF, confirming a diagnosis of neuroangiostrongyliasis. Treatment included glucocorticoid therapy, +/- anthelmintics (fenbendazole) and both dogs made a complete recovery. These are the first confirmed cases of neuroangiostrongyliasis in canine patients in the United States and are also the first dogs anywhere to be diagnosed definitively with A. cantonensis infection based on qPCR testing of CSF.

Keywords: dog, rat lungworm disease, zoonoses

Themes: Veterinary medicine
Dr. Vachel Gay V. Paller is a Professor and University of the Philippines (UP) Scientist III of the Institute of Biological Sciences, University of the Philippines Los Baños (UPLB). She received her doctoral degree from Kobe University's Graduate School of Health Sciences in Japan, with Parasitology as her field of specialization. She has been active in teaching/mentoring, leading research projects and extension works.

In her 28 years of service in UPLB, Prof. Paller has received several awards recognizing her dedication in teaching and active involvement in research and extension works. She was hailed as one of the UPLB Outstanding Alumni in 2014 for having demonstrated outstanding performance in teaching, research, and public service, and as the UPLB Outstanding Teacher in Biological Sciences in 2016.

But what makes Prof. Paller exceptional is her passion to do science to help improve the lives of the Filipino people. She makes sure that her researches reach the grassroots. She conducts various fora to share her research results and inform the locals and stakeholders. She also conducts trainings and workshops for teachers, researchers, farmers, fishermen, elementary schools, and the locals for capacity building. She has several research interests but decided to focus on parasites, as they are considered “neglected”, with only a handful of experts interested in them. Working on parasites entails engagement with marginalized communities and getting one's hands and feet dirty - but she beats the odds. Prof. Paller believes that parasitology is a field that needs more attention, especially in developing tropical countries like the Philippines, where parasitic diseases are widespread but remain undocumented. Despite challenges and difficulties, she continues to teach, conduct research and extension works, and contribute to the alleviation of poverty and decrease parasitic infections in marginalized communities. She is currently a member of the Board of Directors of the Philippine Society of Parasitology.

Intensive farming: implications for zoonotic parasite emergence

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Institute of Biological Sciences
University of the Philippines Los Baños

Philippines is predominantly an agricultural country composed of small farms with a mean area of 3.0 ha per farm. However, agriculture settings are potential source of etiological agents of food borne diseases. Although information regarding foodborne hazards is widely available from many sources, especially regarding hazards such as bacteria and chemical residues, there is limited information on foodborne parasites. Smallholder farmers lack technological know-how about the dynamics of foodborne parasites for control strategies and this is further aggravated by the complacency in standards and policies for food safety. With the focus of new-age farming system shifting to more organic means as opposed to the conventional ways, there is a need to reassess the susceptibility of farm produce to contamination that may have implications on food safety and public health. Several food-borne parasitic zoonoses have been recorded in the ASEAN region. In the Philippines, recent findings revealed that parasite contamination of farm livestock and produce is a serious potential problem as agents of infection to humans and animals in the country. Many are endemic in certain areas of the Philippines because of the habit of consuming raw or partly cooked food from animals and plants. However, more information on the epidemiology, transmission including the animal hosts involved locally are still poorly understood. Our research team has done some works to investigate the extent of parasite contamination in humans, animals and the environment, and the risk factors that have contributed to their transmission. This includes Angiostrongylus sp. transmission dynamics in agriculture settings. Understanding and unravelling these dynamics and processes can aid in the prevention and control of parasite infections in the country. The goal of this paper is to present our recent findings on parasite contamination of farm produce in various local settings in the country and discuss the transmission dynamics and risk factors with implications to food safety and public health. Challenges, research gaps, and opportunities for interdisciplinary research at the local and international levels toward control strategy will also be discussed.
Lorrin Pang, MD, MPH
Health Officer Maui County, Hawaii Dept of Health

- Princeton University, BA (honors), 1975, Chemistry
- Tulane School of Medicine, MD, 1979, Medicine
- Tulane School of Public Health, MPH, 1979, Tropical Medicine

Dr. Pang has about 6 dozen publications in peer reviewed medical journals covering rabies, HIV, malaria, hepatitis E, and most recently, dengue. 2007-9 was selected to America’s Best Doctors Listing. Since 2013 he has 1) become a reviewer for research proposals for US Congress, 2) been consultant to DNDI (international group developing drugs for neglected diseases). Recent interest in cost-benefit analyses of public health interventions.

Recent publications
Coradi de Freitas D, Gomes LT, Fontes CJ, Tauil PL, Pang LW, Duarte EC. Sensitivity of nested-PCR for plasmodium detection in pooled whole blood samples and its usefulness to blood donor screening in endemic area. Transfusion and Apheresis Science. Published online 10 Feb 2014.


An effective electrified metal barrier to control P. martensi

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The semi-slug (Pammarion martensi) is an effective carrier of the parasite Angiostrongylus cantonensis, which is known to cause Rat Lungworm Disease in humans upon ingestion. Several other slugs present in Maui county also are capable of carrying the rat lungworm parasite, such as the Cuban slug, the African snail, and species within the genus Deroceras. Due to the high morbidity of Rat Lungworm Diseases, efforts to control the semi-slug are of high importance. Efforts should focus on preventing semi-slugs from coming into contact with food produced in gardens or farms. To achieve this, barrier materials installed around the perimeter of the garden bed or field have been suggested. Notable examples include copper tape or salt trenches. Chinese slug and snail farmers developed a metal tape that can be electrified (by battery) for keeping their creatures contained. The alternating positive and negative wires on the strip produce a shock when two differing currents are touched simultaneously. Evidence collected in small-scale laboratory experiments concludes that the electrified metal bands are effective at controlling slugs/snails. The slugs/snails are non-lethally shocked and do not successfully crawl across the electrified tape.

Keywords: A. cantonensis, P. martensi, prevention, control

Themes: Epidemiology, control
Randi L. Rollins  
PhD student

- Weber State University, Ogden, Utah, USA, BS, 04/2014, Major: Zoology Minor: Chemistry  
- University of Hawaii at Manoa, Honolulu, HI, USA, MS, In progress, Zoology  
- University of Hawaii at Manoa, Honolulu, HI, USA, PhD, In progress, Zoology

Ms. Rollins became interested in *A. cantonensis* in 2017, and as a new graduate student at UH Manoa in Dr. Rob Cowie’s lab she began to pursue questions about this parasite. Prior to attending graduate school, she studied chemistry and zoology in Utah at Weber State University. Her interest in wildlife and ecology led her to several projects focused on wildlife biology and conservation as an undergraduate. She worked with HawkWatch International in Utah. She started by analyzing the diet and food abundance of Flammulated owls, then went into the field and collected data on American Kestrel nesting ecology for two years, then collected abundance data on Short Eared owls for one season. Ms. Rollins volunteered part time at the Wildlife Rehabilitation Center of Northern Utah and fed, cared for and treated medical needs of birds, marmots, lizards, and other wildlife. Her first research project was in her junior year (2013), and she designed a study to determine the effects of deer repellents on urban and non-urban mule deer. Later that year she spent 1 month in Costa Rica collecting data on nesting behavior and reproductive success of Olive Ridley sea turtles. After graduation, she spent 3 months as a field technician in Italy, developing standard monitoring procedures of 5 saproxylic beetle species for the European Union, to be adopted as official protocol of which all nations would need to complete every 5 years. She worked in a molecular diagnostics lab (Myriad Genetics Inc.) for 3 years, using cutting edge molecular techniques to determine cancer risk in patients. This combination of field and lab work, focused on wildlife conservation and human health, shaped her interest in zoonotic diseases and ultimately, *A. cantonensis* research. In 2018 she conducted fine scale surveys of *A. cantonensis* presence in snail hosts across Oahu, in partnership with the Hawaii State Department of Health. She optimized molecular techniques to handle a large amount of snail tissue, as a way to avoid potential false negatives due to the localization of the *A. cantonensis* larvae in snail tissue. This large dataset provided insight into host and environmental factors that shape the distribution of *A. cantonensis* in the snail host. A publication of results is in review and another publication in prep. The broad range of intermediate host species of *A. cantonensis* interests her, specifically, the factors that drive heterogeneous prevalence and infection intensity in different snail species. She is currently investigating these factors with experimental infections of snail species with different natural infection prevalence. Another interesting aspect to parasite-host relationships is microbes. The microbial environment of the host can have significant effects on the development, reproduction, and survival of the parasite. This has not been studied in the *A. cantonensis* transmission cycle, but she has started a project that compares the microbial environment of the rat, snail and nematode. Results will hopefully be published next year. She also collaborates with faculty at JABSOM, who are involved with the disease aspect of *A. cantonensis*, to understand what factors are driving zoonosis. Collaboration among public health and medical research professionals and ecological and biological researchers is key to providing the best information about prevention of *A. cantonensis* infection to the public, as well as providing the best care for patients who have acquired the disease. She is interested in engaging in projects that facilitate a deeper understanding of what drives disease transmission cycles and zoonosis in the future.

**Recent publications**


Medeiros MCI, Rollins RL, Echaluse MV, Cowie RH. Species identity and size are associated with rat lung worm infection in gastropod hosts. *EcoHealth*. In review.


**Angiostrongylus Cantonensis Infection Prevalence And Intensity In Its Intermediate Gastropod Hosts: The Impact Of Host Species And Environmental Variables**

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Although most gastropod species may be able to harbor *Angiostrongylus cantonensis*, different species exhibit different infection prevalences and infection intensities. Therefore, the zoonotic risk level associated with gastropods is dependent, at least in part, on the species. We studied the possible impact of host species and environment (rainfall, location) on *A. cantonensis* prevalence and intensity among and within intermediate host species. We collected 960 individuals representing 16 gastropod species from 14 sites across a rainfall gradient (566–3861 mm) on the island of O‘ahu, Hawai‘i. Individuals were weighed and measured and samples of foot and mantle tissue were removed for detection of *A. cantonensis* by qPCR. The data were fit to generalized linear mixed models to assess the relationships of infection prevalence and intensity to environmental factors. Prevalence was in general positively correlated with higher rainfall, but the rate of increase varied among species. In infected individuals, infection intensity varied among species but the rate of increase of intensity with rainfall did not differ. An increase in rainfall supports increased transmission and higher density of infectious larvae in intermediate host species. Additionally, we found the importance of certain host species shifted across the rainfall gradient. These results have important implications for public health, as they show that this is a zoonosis with clear patterns across the environment. This information can be used to determine the zoonotic risk of an individual snail and may assist healthcare providers when determining the likelihood of infection in a patient who may have recently ingested a snail, based, along with other information, on the snail species, size and rainfall level at the location of the incident.

**Keywords:** angiostrongyliasis, geography, Hawaii, precipitation, rat lungworm disease, snails

**Species Identity And Host Size Are Associated With Rat Lung Worm Infection In Gastropods**

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Human encounter with gastropods infected with third stage larvae of *Angiostrongylus cantonensis* (rat lungworm) is probably the cause of the majority of cases of neural angiostrongyliasis in Hawaii. *Angiostrongylus cantonensis* exhibits a wide intermediate host breadth extending across the gastropod phylogenetic tree. However, not all hosts are equally suitable, as prevalence and infection intensity vary greatly among species. We addressed this variation in relation to host size by screening 960 individuals representing 16 species from 14 sites on the island of O‘ahu, Hawai‘i. Snails (including slugs) were weighed and measured before foot and mantle tissue samples were removed for *A. cantonensis* detection by qPCR. We used mixed logistic regression models and linear mixed models, respectively, to assess the relationships of prevalence and infection intensity to gastropod size. In total, 182 individuals (19%) of 14 of the 16 species tested positive for *A. cantonensis*. Within species, we found a general positive relationship between infection probability and size. However, individuals of species that only reach a small adult size (< 200 mg) showed a higher probability of infection than small individuals (< 200 mg) of species that achieve a larger adult size. Overall, when individuals of all size classes of all species were compared, large species had an 8.3 times higher probability than small species of being infected. Infection intensity (qPCR CT values) varied among host species, but was unrelated to host size. These results highlight the importance of not only host species but also host size when considering the risk of *A. cantonensis* infection. Smaller snails are more likely to be ingested accidentally. While individuals of large species have a higher probability of being infected, individuals of small species are more likely to cause transmission than small (young) individuals of naturally large species.

**Keywords:** angiostrongyliasis, Hawaii, mathematical models, rat lungworm disease, snails
An ecological study on eosinophilic meningitis caused by *Angiostrongylus cantonensis*

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Climate change and other weather factors are associated with several infectious diseases, but are rarely reported as being associated with nematode infection. Eosinophilic meningitis (EOM) is an emerging disease worldwide caused by the nematode, *Angiostrongylus cantonensis*. It is transmitted through various agents such as snails and slugs. Temperature and rainfall are associated with snail population. There have been no previous studies on the relationship between weather and EOM. This was an ecological study. Numbers of EOM patients and weather data in Thailand’s Loei province from 2006 to 2017 were obtained using a national database. A Spearman correlation was used to explore the relationship between EOM and weather variables. We developed a Poisson time series model combined with a distributed lag model (DLM) for estimating the effects of weather on EOM. We also created an autoregressive integrated moving average with exogeneous variable (ARIMAX) model for predicting future EOM cases over the following 12 months. There were 1126 EOM patients in the study. Among several weather factors, wind was significantly negatively correlated with the number of EOM patients (rs: -0.204, 95% CI: -0.361 to -0.058; p value: 0.014). The ARIMAX(3, 0, 0) model with wind speed as a variable was appropriate for predicting the number of EOM patients. The predicted and actual numbers of EOM patients in 2018 were highly concordant. In conclusion, wind speed is significantly negatively correlated with the number of EOM patients.

**Key words:** Climate change; Predictive model; Snails; Slugs; Weather
Jan Šlapeta
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- University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic, MVDr, 1999, Veterinary Medicine
- University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic, PhD, 2002, Parasitology
- NIH, New York, 2001-2003, Postdoc
- CNRS, France, 2003-2005, Postdoc
- University of Technology, Sydney, 2005-2007, Postdoc
- University of Sydney, Lecturer-Professor, 2007-, Academic

Jan’s recent interest is to understand the role of *Angiostrongylus* in causing disease Sydney, Australia and to enable improved diagnostics, including opportunities for veterinary point-of-care testing. Jan aims to map the diversity of *A. cantonensis* across the global invasive path, using ever cheaper genome sequencing. The ambitious aim is to sequence 100 well morphologically characterized specimens of *A. cantonensis* from all corners of the world, as well as any *Angiostrongylus* spp. he can get hands on – single genes don’t do it these days. So if you work with any get in touch and send them over!

**Recent publications**


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**The Mitochondrial Genome Of Angiostrongylus Mackerrasae Is Distinct From A. Cantonensis And A. Malaysiensis**

Jan Šlapeta1*, Hayley Valentyne1, David M. Spratt1, Malcolm K. Jones1

Page dimensions: 612.0x792.0

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The native rat lungworm (*Angiostrongylus mackerrasae*) and the invasive rat lungworm (*Angiostrongylus cantonensis*) are endemic in Australia. In 1950s, Josephine Mackerras and Dorothea Sandars undertook a series of experiments with parasites from rats caught by the Brisbane City’s rat-gang dogs to discover the life cycle of what they assumed was *A. cantonensis*. Re-examination of the material from Brisbane, Queensland coupled with meticulous experimental infections revealed presence of a previously undiscovered phenotypically different *Angiostrongylus* species in the Australian rat, *Rattus fuscipes*. In 1968, Manoon Bhaibulaya named the new species *Angiostrongylus mackerrasae*. We will explore the recent controversy about the species identity of *A. mackerrasae*. In this presentation we will combine
morphological and molecular tools to re-confirm the existence of A. mackerrasae. Nematodes collected from the lungs of Rattus fuscipes and Rattus rattus conspecific with A. mackerrasae were collected from the type hosts and type locality in Queensland, Australia. Morphologically characterized A. mackerrasae vouchers were characterized using amplification of cox1 followed by generation of reference complete mtDNA. The morphologically distinct A. cantonensis, A. mackerrasae and A. malaysiensis are genetically distinguishable forming a monophyletic lineage. The use of mtDNA via low coverage whole genome next generation sequencing provides data enabling standardised identification of Angiostrongylus spp. including both laboratory strains and field isolates. Beyond species identification the genomic recourse has the potential to be explored for phenotypic trait of A. cantonensis invasive strains and the capacity to establish in new territories. We will finish by a call for global Angiostrongylus voucher repository with the aim of 100 low coverage whole genomes available within next 3 years.

Keywords: Genome, mtDNA, Rattus, taxonomy, Cox1

Themes: (Related Theme)
Kirsten Anna Snook

Research Support

• University of North Carolina at Chapel Hill, BA, 12/1998, English and Film
• Tulane School of Public Health and Tropical Medicine, New Orleans LA, MSPH, 05/2005, Parasitology

Kirsten is a researcher in the Jarvi Lab at the University of Hawaii at Hilo. Her research is on veterinary and human diagnostics of Angiostrongyliasis. She is interested in the intersection of public health and conservation, and earned her MSPH from Tulane University's School of Public Health and Tropical Medicine in Parasitology. Her thesis was funded by a grant from USGS, and examined how aerial spraying of the pesticide Naled to control mosquito populations against the spread of West Nile Virus might affect invertebrate prey of a resident population of endangered red-cockaded woodpeckers. During this research, she conducted the first full year-long invertebrate faunal assessment at Big Branch Marsh National Wildlife Refuge in Lacombe, Louisiana. After receiving her Masters Degree, she worked with Dr. Frank Cogswell at Tulane University on the immune response elicited by malaria and SIV co-infection and on surveillance and testing for the newly emerged disease West Nile Virus (WNV). She helped develop rapid testing methods for the presence of WNV in mosquito bloodmeals. Kirsten moved from Louisiana to Hawaii after Hurricane Katrina in 2007, where she has worked in entomology for Robert Peck at USGS, for Drs. Peter Follett and Nicholas Manoukis at USDA/ARS/PBARC, and in nematology for Dr. Roxana Myers at PBARC. Kirsten has volunteered in Dr. Jarvi's lab since 2010, and became a full-time Jarvi Lab researcher on Angiostrongylus cantonensis in June of 2018.

Recent publications


Jarvi et al. 2019. Validation of a death assay for Angiostrongylus cantonensis larvae (L3) using propidium iodide in a rat model (Rattus norvegicus) July 2019, Parasitology. DOI: 10.1017/S0031182019000908


Serologic detection (ELISA) of A. cantonensis exposure in humans using 31 kDa antigen isolated from Hawai`i Island nematodes

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Angiostrongylus cantonensis is the etiologic agent of neuroangiostrongyliasis (NA) worldwide, and East Hawaii Island is considered the epicenter of the disease in the USA. Antibody-based tests may be useful in detecting exposure, especially when examination of CSF is not possible. A serological test using A. cantonensis 31 kDa antigen was previously developed in Thailand, and demonstrates 100% sensitivity and 100% specificity. While the Thailand-based test has been shown to be effective in Hawaii, regional antigenic differences in the 31 kDa proteins may exist. The use of native antigen in immunological screening may improve results. A previous study with samples from 435 human subjects compared ELISAs using crude antigen from Hawaii nematodes with dot-blot tests using the 31 kDa antigen isolated from Thailand nematodes. The current study reconsented 199 of the original participants to compare their crude/Thai 31 kDa ELISA/dot blot results to ELISA results using the Hawaii-isolated 31 kDa antigen, using the same serum samples. Adult nematodes were collected from wild rats collected in the course of animal studies in East Hawaii and 31 kDa proteins were isolated using SDS-PAGE. Proteins were quantified by bioanalysis. The antigens and testing methods were compared with particular respect to self-reported disease status (clinical or otherwise) and self-reported exposure data.
Real-time PCR detection of Angiostrongylus cantonensis DNA in peripheral blood of domestic animals

Kirsten A. Snook¹, Alfred Mina², Malia Lyons², Sarah M. Ortega³, Lisa Kaluna¹, Lisa Wood³, and Susan I. Jarvi¹

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Angiostrongylus cantonensis can cause neuroangiostrongyliasis (NA) in non-target mammalian hosts including humans, dogs, cats, horses, and rabbits. Definitive diagnosis of NA in domestic animals, as in humans, requires detection of the parasite DNA in cerebrospinal fluid (CSF); however, collection of CSF is not always feasible, lumbar punctures can be expensive, and the procedure is invasive. PCR has been successfully used to detect Angiostrongylus spp. DNA, including A. cantonensis, from peripheral blood samples of dogs, humans, and rats. Blood products (serum, EDTA blood, and whole clotted blood) were collected by licensed veterinarians from domestic animals presenting with NA symptoms and DNA of A. cantonensis was detected in peripheral blood.
Argon Steel  
Research Technician  
- The Evergreen State College, Olympia WA, USA, B.S., 1986, Plant Ecology  
- U. of Hawaii at Manoa, Dept. of Microbiology, M.A., 2009, Virus phylogenetics  
- U. of Hawaii at Manoa, Dept. of Tropical Medicine, Ph.D., 2017, Virology  

My research focus is on pathogens and their vectors with a particular interest in evolutionary phylogenetics. I have extensive prior experience in natural resource management, plant ecology, and mosquito control. My graduate research looked at the role of dengue virus strain variation and its interplay with the evolutionary ecology of the Aedes mosquito vector. Currently I am working in the laboratory of Dr. Susan Jarvi, where I am evaluating commercial and household products for their suitability as produce washes in order to eliminate the threat of infection by Angiostrongylus cantonensis, the rat lungworm, through the eating of contaminated fruits and vegetables.

Recent publications  

Evaluation of prospective vegetable washes for killing A. cantonensis larvae

Argon Steel, John Jacob, Lisa Kaluna, Ina Klasner, Kay Howe, Steven Jacquier, Will Pitt, Robert Hollingsworth, Susan I. Jarvi  
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Humans typically become infected with Angiostrongylus cantonensis upon ingestion of infective third stage larvae found within snails and slugs. In Hawai‘i Island the introduced semi-slug Parmarion martensi has been implicated in an increase in angiostrongyliasis infections with infections primarily associated with the accidental ingestion of snails or slugs, or potentially, from nematodes left behind in the slug’s slime and/or feces, while eating fruits and vegetables. Given the current paucity of treatment options for angiostrongyliasis, prevention of infection is critical. We evaluated more than 40 different treatments in vitro for their ability to kill A. cantonensis larvae with the goal of identifying a safe and effective fruit and vegetable wash. Our evaluation of treatment lethality was carried out in two phases; first using motility as an indicator of larval survival after treatment, followed by use of a propidium iodide staining assay to evaluate larval mortality. Treatments tested included common household products, consumer vegetable washes, and agricultural crop washes. Our results show general patterns: acid solutions exhibited little to no ability to kill third stage A. cantonensis larvae while alkaline solutions were found to be quite effective. Oxidizers, particularly bleach and chlorine dioxide, also showed larvicidal potential. Surfactants, a frequent ingredient in detergents that lowers surface tension, had variable results but dodecylbenzene sulfonic acid as a 70% v/v solution in 2-propanol was very effective, both in terms of the speed and the thoroughness with which it killed A. cantonensis L3 nematodes. On the other hand, our tests showed very little larvicidal efficacy among consumer-grade “veggie washes”, nor on the part of botanical extracts such as those from ginger, garlic or moringa. Thus, our results suggest promising directions for further investigation.

Keywords: Angiostrongyliasis, Infection control, Food safety, Vegetable wash

Themes: (Related Theme)
Sarah Bronwyn Nelson Strong
ND

- Alfred University, Alfred NY, BS, 2003, Biology, with a focus on phytochemistry and medical anthropology
- Bastyr University, Seattle WA, ND, 2007, Naturopathic Medicine

Dr. Sarah Strong is a naturopathic physician who has been practicing in Hilo, Hawaii for over a decade. Originally from upstate New York, she has always had a special interest in the relationship between nature and medicine. In addition to running a collaborative health clinic, she has spent several years teaching various subjects at a Masters level and working for Pacific Quest, a wilderness therapy program for youth and young adults. She enjoys digging in the dirt, cooking with ingredients from her suburban farm, and having adventures with her husband and daughters.

A Clinical Review Of Traditional And Alternative Therapies In The Treatment Of Angiostrongylus Cantonensis Infection

Strong, Sarah Bronwyn Nelson, ND
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Angiostrongylus cantonensis infections, also known as Rat Lungworm (RLW), are becoming more common in the state of Hawai‘i. We have developed protocols for the treatment of acute infections and these therapies are often effective at completely killing this parasite. However, these treatments are not addressing the potential sequelae that occur from the infection, as well as from the die-off reaction related to the actual treatment. Standard guidelines for prevention after possible exposure have been established in other countries but are not the standard of care in the United States, which may also be contributing to the rise in infection rates.

To help individuals infected with Angiostrongylus cantonensis fully recover we must look at not only treating the initial infection but in being more pro-active in diagnosis, prophylactic therapies, and adjunct treatments. These will aid in minimizing long-term damage to the nervous system, as well as other various common sequelae. Ideally, in time, a standard protocol for treatments-preventative, acute, and recovery phase- can be established. Patients who have suffered (and continue to suffer) from this infection can be valuable assets in helping to better understand the efficacy of various treatments for Angiostrongylus cantonensis.

This presentation reviews standard and alternative therapies that have been used in clinical practice (by both myself and colleagues) to see which show promise and which fall short. Can we decrease the number of infections by Rat Lungworm through better patient education and more pro-active prophylactic treatments? Can we begin to develop a universal protocol for clinicians to utilize in order to optimize positive outcomes by reviewing the available literature and clinical findings?

Keywords: Sequelae, treatment, prophylaxis, recovery, universal protocol
Ms. Tagami is an undergraduate student studying cell, molecular, and biomedical sciences at the University of Hawai‘i at Hilo. She’s been an IDeA Network of Biomedical Research Excellence (INBRE) undergraduate researcher for three years. Her research has been focused on angiostrongyliasis, commonly known as rat lungworm disease in Dr. Susan Jarvi’s lab at the Daniel K. Inouye College of Pharmacy. Projects include genetic analysis of nematodes associated with Hawaiian snails and slugs, efficacy of rainwater catchment filters against *Angiostrongylus cantonensis* larvae, mortality rates of *A. cantonensis* following ultraviolet-C (UVC), and the effects of x-ray irradiation on *A. cantonensis* larvae and the semi-slug, *Parmarion martensi*. She is an entomology research lab assistant at the USDA Agricultural Research Service aiding with x-ray irradiation of fruit fly larvae and experiments on the eradication of the coffee berry borer beetle, *Hypothenemus hampei*. She hopes to continue biomedical research while pursuing a M.D. degree to become a pathologist.

Recent publications


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The third larval stage of the parasitic nematode, *Angiostrongylus cantonensis* causes neuroangiostrongyliasis in mammalian hosts, commonly known as rat lungworm disease. The primary mode of infection is believed to be the consumption of *A. cantonensis* infected intermediate hosts such as slugs and snails, however contaminated water, produce, or fomites are also potential transmission routes. Ultraviolet-C light has been effective in the decontamination of water, produce, and fomites by damaging the DNA and causing mortality of pathogens. While no studies have been conducted on the effects of UVC irradiation on *A. cantonensis*, UVC irradiation has been found effective in killing other nematode species. The objective of this study is to observe the mortality rate of the infective L3 stage of *A. cantonensis* after UVC irradiation. *A. cantonensis* L3 larvae were isolated from drowned wild *Parmarion martensi* semi-slugs. Larvae were irradiated at various UVC doses, stained with propidium iodide, and visualized using the Operetta High-Content Imaging System. Results show larval death rate was positively correlated with UVC dose. High doses of UVC irradiation may be a practical disinfection method for fomites, produce, or water in a variety of settings. Low doses of UVC irradiation resulted in slower mortality rates, however the viability and infectivity of these larvae remains unknown.

Keywords: ultraviolet light, mortality, rat lungworm, radiation, decontamination
Hung-Chin Tsai, M.D., PhD.

Attending physician of Infectious Diseases Department

- Infection Control Office Kaohsiung Veterans General Hospital, Director/deputy director, 06/2014, Infection control
- Associate Professor College of Medicine, National Yang-Ming University, Taipei, Associated professor, 02/2016, HIV medicine, parasitology, Infectious Diseases
- Graduate Institute of Medicine, Kaohsiung Medical University, PhD, 06/2007, Parasitology
- Kaohsiung Medical University, M.D, 06/1996, Medicine

Dr. Tsai graduated from Kaohsiung Medical University in 1996 and got his PhD from Graduate Institute of Medicine, Kaohsiung Medical University in 2007. He studied the mechanism of blood brain barrier damage and worked as a research fellow at Saban Research Institute, Children’s Hospital of Los Angeles and University of Southern California, USA from 2005 to 2006. His research interest is the pathophysiology of eosinophilic meningitis, HIV drug resistance, hepatitis and clinical tropical medicine. Dr. Tsai has published more than 100 papers in peer reviewed journals and served as the team leader in the Veterans General Hospitals and University System of Taiwan Joint Research Program Grant.

Dr. Tsai currently serves as the attending physician of Infectious Diseases Department, and director/deputy director of the Infection Control Office, Kaohsiung Veterans General Hospital. He is also the Director for Taiwan Infection Control Society/Taiwan AIDS Society.

Recent publications


Tsai HC, Chen YH, Yen CM, Lee SS, Chen YS. Increased 14-3-3β and y protein isof orm expressions in parasitic eosinophilic meningitis caused by Angiostrongylus cantonensis infection in mice. PLoS One. 2019;14:e0213244

Tsai HC, Chen YH, Yen CM, Chung LY, Wann SR, Lee SS, Chen YS. Dexamethasone downregulates the expressions of 14-3-3β and y protein isof orms in mice with eosinophilic meningitis caused by Angiostrongylus cantonensis infection. Korean J Parasitol 2019 in press
Dexamethasone downregulates the expressions of MMP-9 and oxidative stress in mice with eosinophilic meningitis caused by *Angiostrongylus cantonensis* infection

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Matrix metalloproteinase-9 (MMP-9) has been found to induce blood–brain barrier leakage in mice and human with eosinophilic meningitis caused by *A. cantonensis* infection. Oxidative stress was also found in the CNS of mice infected with *A. cantonensis* during days 12–30 after infection due to reactive oxygen species (ROS) overproduction in CSF. Steroid has been found to be beneficial in patients and mice with eosinophilic meningitis caused by *A. cantonensis* infection, but how it produces the effect is not known. We speculated that the effect of steroid in eosinophilic meningitis caused by *A. cantonensis* infection was mediated by down regulation of MMP-9 and oxidative stress pathway via glucocorticoid receptors (GRs).

In this study, we found that blood brain barrier dysfunction occurring in mice with eosinophilic meningitis 2-3 weeks after infection as evidence by the increased extravasation of Evans blue and CSF albumin levels. Administration of dexamethasone significantly decreased the amount of Evans blue and CSF albumin. The effect of dexamethasone was mediated by the GRs and heat shock protein 70, resulting in the subsequently decreased expression of NF-κB, p-JNK and JNK in the CSF and brain parenchymal after 2 weeks steroid administrations. Steroids also decreased CSF/brain homogenates MMP-9/TIMP-1 expressions, but had no any effect on the CSF MMP-2 levels, indicating that the MMP-9 instead of MMP-2 playing the major roles in BBB dysfunction in mice with eosinophilic meningitis. The 8-OHdG concentration was increased gradually after 1-3 week infections and the administration of dexamethasone significantly down-regulated the oxidized derivative 8-OHdG concentration in CSF. In conclusion, increased 8-OHdG and MMP-9 concentration was found in mice with eosinophilic meningitis caused by the *A. cantonensis* infection. The effect of dexamethasone was mediated by the GRs and significantly decreased not only the levels of 8-OHdG and MMP-9, but also NF-κB, p-JNK and JNK.

**Keywords:** *Angiostrongylus cantonensis*, corticosteroid, eosinophilic meningitis, oxidative stress.
Heather D S Walden
Assistant Professor of Parasitology

- University of Kentucky, Lexington, KY, B.S., 08/1999, Biology
- Appalachian State University, Boone, NC, M.S., 05/2004, Biology/Genetics
- Auburn University, Auburn, AL, Ph.D., 05/2008, Biomedical Science/Parasitology

Dr. Walden’s parasitology laboratory is focused on zoonotic parasitic disease, diagnosis and classical parasite biology, including modes of transmission and pathogenicity in the definitive host. The majority of her research and collaborations have spanned several parasite species and have involved human and veterinary parasitic infections throughout the United States, Mexico, Ecuador, the Galapagos Islands, and the African nations of Zambia and Senegal. The parasitology laboratory regularly works with parasites of exotic and domestic hosts, as my classical and molecular training allows inclusion of internal and external parasites of all taxonomic groups ranging from fish, amphibians, reptiles and birds to mammals, including marine mammals and nonhuman primates. We are able to identify parasites of exotic definitive and intermediate hosts, which has led to the discovery of parasites new to the United States and Florida, as well as descriptions of parasite species found in new hosts. Current work includes zoonotic metastrongyloid nematodes, specifically *Angiostrongylus cantonensis*, and the geographic distribution and host utilization throughout Florida. The ongoing goal of the laboratory is to sustain the *A. cantonensis* lifecycle to allow further, more in depth analysis of infection and potential hosts of this parasite.

**Positions and Employment**

- 2002-2004 Genetics Laboratory Teaching Assistant, General Biology Teaching Assistant, Appalachian State University, Boone NC
- 2004-2008 Graduate Research Assistant, Auburn University, College of Veterinary Medicine, Auburn AL
- 2008-2010 Biological Scientist, University of Florida, College of Veterinary Medicine, Gainesville FL
- 2010-2015 Research Assistant Professor of Parasitology, University of Florida, College of Veterinary Medicine, Gainesville, FL
- 2015-present Assistant Professor of Parasitology, University of Florida, College of Veterinary Medicine, Gainesville, FL

**Professional Societies and Honors**

- 2004 – 2008 Southeastern Society of Parasitologists (SSP), member
- 2004 – present American Society of Parasitologists (ASP), member
- 2004 – present American Association of Veterinary Parasitologists (AAVP), member
- 2015 – present AAVP Education committee member
- 2016 – present Companion Animal Parasite Council (CAPC), parasitologist board member
- 2016 – present American Heartworm Society, member
- 2016 Zoetis Distinguished Veterinary Teacher Award
- 2017 – present University of Florida Emerging Pathogens Institute, faculty member
- 2018 – present CAPC board treasurer

**Contributions to Science (a selection of publications)**


Angiostrongylus cantonensis in Florida, USA: current status

Heather D. S. Walden¹, John Slapcinsky², & James F. X. Wellehan, Jr.¹

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²Florida Museum of Natural History, Gainesville, Florida, 32611, USA

Angiostrongylus cantonensis has been found in Florida, USA, from the panhandle in the north to Miami and surrounding areas in the southern parts of the state, in both definitive and intermediate hosts in a limited study completed in 2015. Intermediate hosts included both native and non-native gastropod species with new host species recorded. Many areas in our survey with higher A. cantonensis prevalence were those with a high human population density, which suggests it is a matter of time before human infections occur in Florida. Case reports in the state currently involve non-human primates and include a gibbon and orangutan in Miami and non-reported capuchin monkey and red ruffed lemur in Gainesville. Current status of A. cantonensis in Florida will be discussed, including studies anticipated for the future.

Keywords: rat lungworm, Florida, rats, Bradybaena similaris, Zachrysia provisoria

Themes: Geographic distribution of A. cantonensis
Matthew Wun
Veterinary student

- The University of Sydney, Australia, BVetBiol, 12/2015, Veterinary science
- The University of Sydney, Australia, DVM, 12/2019, Veterinary science

Matthew is a final-year vet student at the Sydney School of Veterinary Science with an interest in both basic and clinical research. During his degree, he has won research scholarships at the Sydney Pharmacy School and Cornell Leadership Program for Veterinary Students. He is currently involved in a number of rat lungworm projects in association with Richard Malik.

1. Correlated Gross, Histologic and Imaging Findings in Adult Wistar Rats Chronically Infected with Angiostrongylus Cantonensis

Matthew K. Wun¹, Sarah Davies², Derek Spielman¹, Rogan Lee³ & Richard Malik⁴

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While the gross and microscopic changes caused by Angiostrongylus cantonensis in the thorax of rats have been elegantly described in the classic studies of Josephine Mackerras, their appearance with modern diagnostic imaging techniques has not been reported. This project correlates gross pulmonary, vascular and cardiac changes with the corresponding microscopic alterations using observations from radiology, computed tomography (CT), CT angiography, echocardiography and histological examinations in mature Wistar rats (R. norvegicus) chronically infected with moderate burdens of A. cantonensis. Plain radiography (dorsoventral and lateral orthogonal views), CT and CT angiography showed a moderately severe alveolar pattern preferentially affecting caudal portions of the left and/or right caudal lung lobes, associated with dilatation of the caudal lobar pulmonary arteries. Presumptive worm profiles were visualised by echocardiography in the right ventricular outflow tract just below the level of the pulmonary valve or straddling either the pulmonary and/or the tricuspid valves in rats immobilised by gaseous (isoflurane) or injectable (midazolam/ketamine) anaesthesia. Extensive, multifocal coalescing areas of dark discolouration and multiple pale foci affecting one or both caudal lung lobes were observed at necropsy. Histologically, these were comprised of numerous large, confluent granulomas (often centred on egg nests) and fibrotic nodules. At necropsy, adult male and female lungworms were found predominantly in the mid- to distal pulmonary arteries, especially those subserving the caudal lung lobes. Often there was segmental aneurysmal dilatation of portions of the pulmonary arteries were a mass of worms was found to be present. These gross, histopathologic and radiological changes are comparable to those described in dogs with Angiostrongylus vasorum infection, suggesting rats infected with A. cantonensis may be a viable model for A. vasorum infection in the dog.

Keywords: Angiostrongylus cantonensis; rats; radiology; computed tomography; ultrasonography; pathology

Themes: Parasitology, Veterinary, Imaging, Pathology

2. Magnetic Resonance Imaging Findings In 5 Dogs With Neuroangiostrongliasis

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MRI images were obtained, using a high field (1.5 Tesla) unit in 4 instances and a low field (0.3 Tesla) unit in the remaining case, as part of the investigation of five canine patients. In three of these, neuroangiostrongliasis was considered the most likely diagnosis, but various considerations gave cause for the attending clinician to obtain neuroimaging as well as CSF for laboratory analyses under the same general anaesthetic. In the remaining two cases, signs of encephalitis eclipsed those referable to spinal cord dysfunction and MRI imaging was considered essential to fully characterize the problem. Sagittal T1 and T2 weighted images were obtained of the spinal cord and/or brain, together with supplementary Short-TI Inversion Recovery (STIR) or Fluid-attenuated inversion recovery (FLAIR) images, as appropriate, followed by post-gadolinium T1-weighted or FLAIR images. In these five representative dogs with neural angiostrongliasis, the three dogs with preponderant spinal and radicular signs had scans showing mild diffuse enlargement of variable portions of the spinal cord (cervical in one dog, thoracolumbar in two others), with small areas
of patchy T2 hyperintensity and patchy enhancement after paramagnetic contrast. In the two dogs with encephalitic signs there was increased contrast enhancement of the meninges through both cerebral cortices, accompanied by increased contrast enhancement of the meninges surrounding the spinal cord. In the dog which developed irreversible central blindness, there was prominent involvement of the occipital cortex. There was invariably evidence for meningeal involvement even when it was not prominent clinically.

**Keywords:** *Angiostrongylus cantonensis*; dogs; magnetic resonance imaging (MRI), radicular, encephalitic

**Themes:** Parasitology, Veterinary, Imaging